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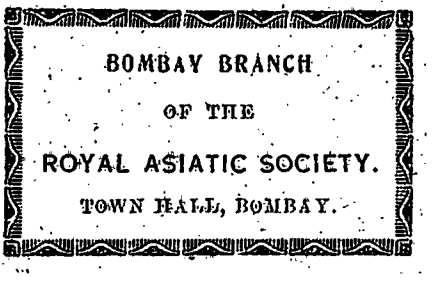

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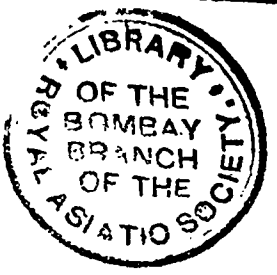

REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1945.

134336

1945 to 1947

Issued under the authority of the Governing Body,
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With the compliments of
Colonel E. Cotter, C.I.E., V.H.S.,
I.M.S., Secretary, Governing Body
and Scientific Advisory Board,
Indian Research Fund Association.

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The Scientific Advisory Board consisted of the following :—

Chairman :

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Members :

Colonel E. Cotter, C.I.E., I.M.S., Public Health Commissioner with the Government of India (1st January to 31st March, 1945). *Secretary.*

Lieut.-Colonel C. A. Bozman, I.M.S., Offg. Public Health Commissioner with the Government of India (1st April to 31st December 1945). *Secretary.*

Lieut.-Colonel H. W. Mulligan, I.M.S., Director, Central Research Institute, Kasauli.

Rai Bahadur Dr. B. M. Das Gupta, Director, School of Tropical Medicine, Calcutta (1st January to 15th April, 1945).

Lieut.-Colonel C. L. Pasricha, I.M.S., Director, School of Tropical Medicine, Calcutta (16th April to 31st December, 1945).

Lieut.-Colonel S. S. Sokhey, I.M.S., Director, Haffkine Institute, Parel, Bombay.

Dr. C. G. Pandit, O.B.E., M.B.B.S., Ph.D., D.P.H., D.T.M., Director, King Institute, Guindy, Madras.

Major-General G. Covell, C.I.E., I.M.S., Director, Malaria Institute of India, Delhi.

Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S., Director, Drug Research Laboratory, Jammu, Kashmir (1st January to 17th March, 1945).

Dr. V. R. Khanolkar, B.Sc. (Lond.), M.D. (Lond.), Director of Laboratories, Tata Memorial Hospital, Bombay (18th March to 31st December, 1945).

Dr. A. N. Goyle, M.D., Ph.D., Professor of Pathology, King Edward Medical College, Lahore.

Dr. V. N. Patwardhan, M.Sc., Ph.D., Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Co-opted member :

Lieut.-Colonel C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene and Public Health, Calcutta (30th November to 31st December 1945).

Composition of Advisory Committees for the year 1946.

1. *Cholera.*

Lieut.-Colonel H. W. MULLIGAN (*Chairman*).
THE PUBLIC HEALTH COMMISSIONER WITH THE GOVERNMENT OF INDIA.
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Major J. H. GORMAN.
Dr. C. G. PANDIT.
Dr. S. R. PANDIT.
Lieut.-Colonel C. L. PASRICHA.
Rao Bahadur Dr. K. C. K. E. RAJA.
Lieut.-Colonel S. S. SOKHEY.
Lieut.-Colonel M. L. AHUJA.
Dr. K. V. VENKATRAMAN. } (*Joint Secretaries*).

2. *Malaria.*

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Lieut.-Colonel M. K. AFRIDI.
Dr. B. ANANTHASWAMY RAO.
Rai Bahadur Dr. A. C. BANERJEA.
Bt.-Colonel Sir RAM NATH CHOPRA.
Rai Bahadur Dr. B. M. DAS GUPTA.
Lieut.-Colonel H. W. MULLIGAN.
Dr. D. K. VISWANATHAN.
Lieut.-Colonel JASWANT SINGH (*Secretary*).

3. *Nutrition.*

THE PUBLIC HEALTH COMMISSIONER WITH THE GOVERNMENT OF INDIA (*Chairman*).
Professor S. P. AGHARKAR.
Dr. K. P. BASU.
Khan Bahadur Dr. A. H. BUTT.
Sir THEODORE GREGORY.
Dr. B. C. GUHA.
Dr. V. N. PATWARDHAN.
Dr. G. SANKARAN.
Mr. J. SARGENT.
Mr. H. R. STEWART.
Major G. WILLIAMSON.
Dr. W. R. AYKROYD (*Secretary*).

4. *Plague.*

Dr. V. R. KHANOLKAR (*Chairman*).
Dr. A. N. GOYLE.
Dr. C. G. PANDIT.
Dr. S. R. PANDIT.
Dr. K. V. VENKATRAMAN.
Lieut.-Colonel S. S. SOKHEY (*Secretary*).

5. *Clinical Research.*

Sir LAKSHMANASWAMI MUDALIAR (*Chairman*).
Lieut.-Colonel A. T. ANDREASEN.
Dr. M. N. DE.
Lieut.-Colonel H. K. GOADBY.
Dr. A. N. GOYLE.
Dr. S. W. HARDIKAR.
Lieut.-Colonel JASWANT SINGH.
Dr. M. G. KINI.
Dr. P. KUTUMBIAH.
Major F. SHEPPARD.
Captain E. J. SOMERSET.
Dr. V. R. KHANOLKAR (*Secretary*).

6. *Maternity & Child Welfare.*

Dr. J. JHIRAD (*Chairman*).
Dr. M. I. NEAL EDWARDS.
Lieut.-Colonel H. M. LAZARUS.
Dr. JEAN ORKNEY.
Dr. S. PANDIT (*Secretary*).

7. *Rabies.*

Major-General G. Covell (*Chairman*).
Lieut.-Colonel M. L. AHUJA.
Lieut.-Colonel H. W. MULLIGAN.
Dr. S. R. PANDIT.
Dr. S. R. SAVOOR.
Dr. N. VEERARAGHAVAN.
Dr. A. G. BROOKES (*Secretary*).

8. *Industrial Health.*

THE PUBLIC HEALTH COMMISSIONER WITH THE GOVERNMENT OF INDIA (*Chairman*).

Mr. S. C. JOSHI.
Major E. L. JONES.
Lieut.-Colonel C. K. LAKSHMANAN.
Dr. J. M. ORKNEY.
Dr. V. N. PATWARDHAN.
Mr. M. T. RAJU.
Dr. J. N. RAY.
Dr. M. N. RAO (*Secretary*).

The Board held its annual meeting on the 30th November and 1st December, 1945, and confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers and decided on the co-option of experts for its annual meeting. It also considered in detail the programme of research for the year 1946-47 and recommended the programme given on pages 160–164 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1945.

The researches, which were carried out during the year under report were recommended by the Board at its meeting held in New Delhi on the 28th and 29th November, 1944 and were approved by the Governing Body of the Indian Research Fund Association at its meeting held in New Delhi on the 17th March, 1945.

CHOLERA

1. Cholera treatment unit under the Director, School of Tropical Medicine, Calcutta.

This unit was fully staffed on 16-4-45. The work carried out by this unit is summarized below :—

(A) *Field work.*—A mobile unit went to Faridpur on 19-4-45 and stayed there for 8 days. Great difficulty was experienced in ' catching-up ' with reported attacks of cholera as often the disease had subsided by the time the treatment unit arrived. Eighteen cases of cholera were treated; 3 of the 9 treated with sulphaguanidine and 6 of the 9 controls died.

(B) *Selection of cases for trial.*—This was based on alternative cases being admitted to hospital.

Patients treated with sulphaguanidine.—Sulphaguanidine was issued as powder in paper packets containing 3 grammes of the drug. One packet was suspended in an ounce of water and given every four hours for 3 days, and then twice a day for three days. Controls received an inert powder.

Such cases which died or absconded within 16 hours of admission to the hospitals were excluded. This was done in both the series (36 from the sulphaguanidine series and 34 from the control series) and the results of this trial are summarized below :—

	Treated with sulphaguanidine	Control series (not treated with sulphaguanidine)
Total number of clinical cases of cholera	456	457
Number of deaths irrespective of cause	11 or 2·1%	40 or 8·77%

The results are statistically significant (the odds against chance being 1600 to 1).

(C) *Trial of sulphadiazine in cholera.*—Sulphadiazine was given to a series of cases and its value compared with sulphaguanidine. Sulphadiazine

dose :—1 gramme every four hours for two days and then three times a day for one day.

	Treated with sulphaguanidine	Treated with sulphadiazine.
Total number of clinical cases of cholera	144	144
Number of deaths irrespective of cause	2 or 1·39%	9 or 6·25%

The work done this year has shown that sulphaguanidine is of value in the treatment of cholera. Unfortunately, however, the enquiry was started at a time when the cholera epidemic was already at its height.

Preliminary work on the chemistry of cholera stools has given interesting results.

(D) *Examination of the vomit and stools of cholera patients.*—Both the vomit and the stools are hypotonic, average being 0·5 gramme per 100 ml.

Unfortunately this work was undertaken at a time when the cholera epidemic had declined and the cases were atypical.

2. Cholera treatment unit under the Director, King Institute, Guindy, Madras.

The staff sanctioned by the Governing Body of the Indian Research Fund Association to undertake investigation in the treatment of cholera was partially assembled in June 1945. In the absence of definite outbreaks of cholera in the districts which could be taken up for investigation, the staff was engaged on cholera work in the Institute itself, and a detailed bio-chemical study of normal human blood was made for the purpose of training the staff in the conduct of these tests when suitable cholera cases were available for study.

The technique of determining the specific gravity of blood and plasma by the copper sulphate method and the bio-chemistry of normal human blood and serum were also undertaken.

Up to the time of the report no cases were available for study both in the field and in the city of Madras.

Laboratory studies :—

Earlier in the year, however, 455 strains of Vibrio cholerae were isolated. All these belonged to the OGAWA sub-type of Vibrio cholerae, excepting one which was isolated recently from a village in Kistna district which proved to be of INABA type. It is interesting to note that this strain has been isolated after a period of nearly two years, the previous isolation of this sub-type being from Madura district in February 1944.

Work on the following lines is proceeding :—

(1) Nutritional requirements of V. cholerae are being studied with a view to isolate a growth activating factor from peptone.

(2) An acid hydrolysate of casein was prepared and its property studied from the point of view of growth promoting substances.

(3) Reilley's medium was also studied in a similar manner and it was noted that successful isolations could be made with this medium in the presence of coliform and other organisms.

(4) The preparation of mannose from ivory nut powder obtained from America some time ago is in progress.

3. Enquiry to prepare monospecific high titre cholera sera under the Director, King Institute, Guindy, Madras.

This enquiry was started with the object of providing monospecific type sera for the diagnosis of sub-types of cholera vibrios to various workers in India. 34 rabbits and 2 goats are under immunisation and it is expected that the serum from these will be available for issue in the near future.

4. Enquiry on the immuno-chemical behaviour of *V. cholerae* under the Director, Central Research Institute, Kasauli.

The work of this enquiry was commenced in July, 1945, in response to a recommendation made by the Cholera Advisory Committee of the I.R.F.A. The intention is that this enquiry will be absorbed into a new cholera enquiry at the end of the current year.

Progress has been slow owing to the inability to procure certain essential items of equipment and chemicals, but these are expected to become available shortly. Attention has been directed chiefly to preliminary work including the raising of high titre precipitin sera from the Inaba and Ogawa sub-types of *V. cholerae* and the preparation of samples of the polysaccharids from selected strains grown on Douglas trypsin digest broth and other liquid media.

5. Enquiry to test the validity of the data collected by the I.R.F.A. enquiry into the statistical evaluation of anti-cholera inoculation under the Director of Public Health, Madras and Dr. C. Chandrasekaran, All-India Institute of Hygiene and Public Health, Calcutta.

Introduction. In August 1944, Dr. R. Adiseshan, the then Director of Public Health, Madras, submitted to the Indian Research Fund Association, a report on statistical evaluation of anti-cholera inoculation as a personal prophylactic against cholera and its efficacy in the prevention and control of cholera epidemics. As the reliability of the basic data compiled for the report was questioned, an enquiry was set up with a view to ascertain the correctness of the basic data. Dr. Chandrasekaran, of the All India Institute of Hygiene and Public Health, Calcutta, who investigated this question was of opinion that, in a random sample of villages, the data relating to which were examined by him, the maximum possible error found was in the neighbourhood of 7 per cent, and that this error when applied to the whole data would not statistically invalidate the conclusions reached in Dr. Adiseshan's report. Nevertheless, he

considered that further statistical enquiry on the lines suggested by him should be carried out in 70 to 80 *cheris* in South Arcot district, which had two outbreaks of cholera in 1942-43. The Indian Research Fund Association accordingly proposed that the Director of Public Health, Madras, in collaboration with Dr. Chandrasekaran should conduct the enquiry.

Method of enquiry. The steps that should be taken to test the validity of the data collected in Dr. Adiseshan's enquiry were drawn up by Dr. Chandrasekaran. The method of the present enquiry is similar to that of Dr. Adiseshan (Vide report of the Scientific Advisory Board for 1944, pages 4 to 21) the main departure being the use of smaller units to restrict the zone of infection. The *cheri* is now being taken as the unit instead of the village or hamlet as was done previously, and about 70 to 80 *cheris* that suffered more than one outbreak during the cholera epidemic in 1942-43 in the South Arcot district are to be included in the present enquiry. The data for these *cheris* are for the most part extracted from those collected in the original enquiry. The population of the *cheris* are, however, actually enumerated by fresh field work and the original classification of the cholera cases in the *cheri* into inoculated and not inoculated are also checked carefully by a reference to the inoculation list.

Dr. Chandrasekaran personally instructed the field staff, regarding the method of enquiry and the actual enquiry was started on 13-9-1945.

Enquiry. The necessary basic data relating to the *cheris* have to be extracted from (i) the village cholera registers, and (ii) the registers of inoculations done in the village. The former are maintained by the village headmen and are readily available being permanent records. The inoculation registers which have not been standardised are with the Health Inspectors concerned, and have not been prescribed as permanent records. Hence, before a village is visited, these two records should be completely available. In the four ranges visited so far, inoculation registers of 13 *cheris* (4 in Cuddalore range, 3 in Kattumannarkoil range, 5 in Chidambaram range, and 1 in Tindivanam range) were not available, and enquiry in respect of these *cheris* had to be abandoned. 18 *cheris* in all were visited till the end of November 1945 by the field staff. No anticipatory inoculations were done in four of these, leaving 14 *cheris* which have yielded figures for analysis.

Cholera epidemic registers. In cholera epidemic registers, provision is made for entering the names of persons attacked and the residence in addition to other particulars such as age, sex, etc. In 12 out of 14 registers, the names of fathers or husbands are also given. This additional information has largely helped to identify the persons easily. In the case of two other registers, these additional particulars are not given, but all except one out of 23 cases in these two *cheris* were identified by the village headmen.

The classification of attacks according to the inoculated and the uninoculated was done by the health staff in all villages, except one, (Vakkaramari), after varying intervals of time. It is understood that this classification was done by them with reference to the inoculation registers. The village headmen of these villages stated that they did not receive the circular dated 23rd November 1942 of the Collector of South Arcot district, asking them to enquire and enter whether a case of attack was inoculated or not. However, the inoculation registers were scrutinised to see whether each case of attack was inoculated or not. The names of six persons who were attacked and classified as uninoculated by the health staff have been traced in the inoculation registers.

It is understood from enquiry that all cases of cholera which occurred in the villages might not have been recorded by the village headmen. Cases of omission to enter attacks of cholera especially when the health staff are not present throughout the epidemic are not uncommon. At this distance of time it is not possible to verify whether all cases have been recorded.

In all the villages visited so far, no culture was sent for examination to establish cholera infection in the villages. This is important, especially in villages where there were one or two cases only in an outbreak. In 4 *cheris* out of 14, there was only one case in the second outbreak and in another, there were two cases only.

Inoculation register. The inoculation registers were maintained both in printed and manuscript forms. In the printed forms, provision is made for entering name of person inoculated, address, age, sex, caste, and dosage of vaccine. Inoculation registers in respect of 4 *cheris* were completely maintained in printed forms, and in respect of 10 *cheris* in manuscript form alone, or in manuscript and printed forms. In the case of manuscript forms, where no particulars other than name and age are given, it was difficult for the village headmen and even *Talayaris*, who are village menials and who reside in *cheris*, to identify the persons in the registers. Sometimes, the help of other elderly men in *cheris* had to be requisitioned to identify the persons, and their version had to be accepted. In all the 14 *cheris*, 85 persons out of 1,369 inoculated could not be identified in spite of intense enquiry.

In all the 14 *cheris* surveyed, 63 persons belonging to *cheris* of different villages were inoculated and their names recorded in inoculation registers of the *cheri* under survey. Similarly, it is quite possible that some people of these *cheris* might have been inoculated in other villages, especially if the *cheris* are situated close to big towns, like Nellikuppam or close to other villages. Cases occurring among these will be put down as uninoculated, inasmuch as their names would not be found in the inoculation register of the *cheri*. Every effort was made in all cases of attacks to properly classify them by personal enquiries whether they were inoculated or not. In some cases, persons concerned do not remember about the inoculation.

Dosage. Although a column has been provided for entering the dosage given in the printed inoculation registers, record of dosage of cholera vaccine administered has not generally been made in the registers of villages under report. In one out of fourteen villages included in this report, and in six others surveyed after the period to which this report relates, dosages of cholera vaccine administered have been entered. It is seen that the dosages varied from 0·1 c.c. to 1·0 c.c., and that no uniform dosage for persons of the same age and sex was adopted. The question as to how far this factor will affect the usefulness of the data collected is being separately examined.

Population of cheris. As regards estimate of population of the affected *cheri* during the epidemic of 1942-43, actual enumeration of persons now living in each hut of the affected *cheri* was made. At the same time, enquiries about the new residents after the period of epidemic, and about residents during the epidemic, but who have since left, were made and necessary corrections were applied to the total population enumerated in the *cheri* to arrive at the population of the *cheri* exposed to risk during 1942-43. In this connection, the unprotected persons registers of villages containing names of persons in each hut in the *cheri* have been helpful.

Analysis of data collected. The enquiry has been completed in 14 *cheris* with a total population of 3,934, of whom 1,101 were inoculated between the onset of the first outbreak and six days prior to the second outbreak, and 183 were inoculated during six days prior to the second outbreak, and during the second outbreak. In the first outbreak, there were 62 attacks of whom 47 died, and in the second 68 attacks of whom 42 died.

The data available at present are too few for a detailed analysis on the lines suggested by Greenwood and Yule. A preliminary analysis of the efficacy of anticipatory inoculation is given below utilising the data for the second outbreak, and, considering inoculations done between the onset of the first outbreak and 6 days prior to the second as anticipatory inoculations. The basic data are :

Total number of persons who had anticipatory inoculations ..	1,101
Attacks among the protected in the second outbreak	13
Attack rate per mille of the protected	11·8
Total number of uninoculated persons (excludes 47 deaths from cholera in the first outbreak)	2,603
Attacks among the uninoculated in the second outbreak ..	55
Attack rate per mille of the uninoculated	21·1

The attack rate among the uninoculated is about 1·8 times the rate among the protected group. The Chisquare test for the significance of the difference in the two rates as applied to the following four-fold table :

	Attacked	Not attacked	Total
Protected ..	13	1,088	1,101
Uninoculated ..	55	2,548	2,603
Total ..	68	3,636	3,704

gives a value of 3·69 which is slightly lower than the significance level at 5%, viz., 3·84. The difference in rates, therefore, is approaching statistical significance.

The investigation is being continued in other *cheris* taken up for investigation, and, the result of final analysis after the enquiry is completed may be awaited.

MALARIA

6. Malaria Institute of India (Research Section), Delhi and Insecticide and Mosquito Repellent Enquiry under the Director, Malaria Institute of India, Delhi.

The research activities of the Institute were again severely curtailed due to the abnormal conditions prevailing on account of the War. The post of Malaria Research-Officer remained unfilled and a large proportion of the staff was employed in connection with instructional courses in malariology for officers deputed from the various branches of the Defence Department, Railways, etc. Six such courses were held during the year and a total of 147 officers and anti-malaria assistants were trained. Special training in Anti-malaria Measures including preparation and application of D.D.T. was arranged for 9 supervisors of various ordnance factories, 5 sanitary inspectors, 1 malaria inspector, 1 overseer, 1 malaria supervisor of a coalfield, 2 members of Bhopal state forces, 2 officers of the Public Health Department, Punjab and 2 from the U.P. Several other workers including 2 Chinese were also afforded facilities in the studies of Malaria.

Publications.

Journal of the Malaria Institute of India.

Two numbers, containing 13 papers and 4 abstracts, have been published.

Scientific material sent to other workers.

A strain of *P. cynomolgi* and J.S.B. stain were supplied to the Mammalian Malaria Enquiry. J.S.B. stain was also supplied to Dr. Reginald D. Manwell of New York. Slides showing different stages in the development of human malaria parasites both in man and mosquito, pinned specimens of mosquitoes, Gambusia fish and J.S.B. stain were supplied to a number of workers in India. Nozzles for use with D.D.T. and Mish hand sprayers were sent to various workers in India, Australia and Africa.

Testing of insecticides.

Work on the development of a D.D.T. emulsion concentrate prepared with turpentine oil and medium kerosine extract (MKE) was continued. With the apparatus designed at the Institute 5 or 6 persons can prepare 120 gallons of emulsion concentrate in 2½ hours.

The study of different types of equipment for the application of D.D.T. has been continued. For indoor spraying a stirrup pump fitted with a special lance and nozzle designed at the Institute has been found satisfactory. With this apparatus 5 persons can spray 56 rooms of the size of the average village hut or menial quarter in 2½ hours. A pressure regulating valve to minimise the risk of the bursting of the hoses has also been designed.

A series of field experiments with both oily solutions and watery emulsions of D.D.T. have been carried out in certain villages in the vicinity of Delhi and also at Siliguri in the Bengal Duars. D.D.T. has been shown to be extremely effective against *A. mininus*, the chief malaria carrier of the foothills of Bengal, Assam and Burma, the residual effect persisting for more than 2 months. In

the vicinity of Delhi where *A. culicifacies* is the vector, the results have been less spectacular, the residual effect being much less persistent. Outdoor spraying in the Delhi area has not proved successful. On the whole, watery emulsions of D.D.T. have been found to be more effective than oily solutions for spray killing adult mosquitoes. This is in conformity with the results reported by other workers.

The effect of D.D.T. emulsions and oily solutions on the aquatic stages of mosquitoes and on other aquatic fauna has also been studied.

Biological tests have been carried out on a number of insecticides received from various sources, including freon bombs charged with pyrethrum extracts and pyrethrum plus D.D.T. The sparklet bulb type of dispensar was also tested and was found unsatisfactory in that there were marked variations in the degree of atomisation and spraying time between individual bulbs.

Entomological research.

The study of mosquito bionomics has been continued. Colonies of *Aedes aegypti* and *Aedes vittitis* and *Anopheles stephensi* have been maintained in the laboratory for this purpose.

Field researches.

Epidemiological studies have been continued in certain villages in the vicinity of Delhi. Antimalaria operations in Delhi have been directed and supervised as in previous years. D.D.T. has been used in this area for the first time both from the ground and from aircraft. The malaria incidence has been lower in proportion to the population at risk than in any previous year for which records are available.

Laboratory researches.

A number of monkey malaria strains have been maintained. High titre anti-human and anti-bovine sera have been prepared and approximately 900 precipitin tests of mosquito blood meals carried out on specimens sent from many different parts of India.

A series of experiments with a new synthetic antimalarial drug, Paludrine (M 4888) prepared by Imperial Chemical Industries Ltd., has been carried out on monkeys infected with malaria. This has proved to have marked plasmodicidal effect comparing favourably in all respects with mepacrine. A number of other drugs for which antimalarial properties had been claimed were tested, but none of these proved to have any plasmodicidal value.

A new blood stain which can be used as a substitute for Leishman's or Giemsa's stains and which is more easily prepared from ingredients obtainable in India has been developed.

Southern India branch, Coonoor.

A series of investigations has been carried out with regard to the bionomics of *A. fluviatilis*, including observations on the time of entry into houses, time of feeding, size of blood meal and its influence on ovarian development, time of oviposition and the behaviour of gravid females under varying conditions and duration of the gonotrophic cycle.

A strain of *P. gallinaceum* has been maintained and colonies of *A. stephensi* and *A. fluviatilis* have been carried on in the laboratory.

Experiments have also been carried out with reference to the technique of rearing larvae.

INSECTICIDE AND MOSQUITO REPELLENT ENQUIRY.

A series of field experiments with D.D.T. has been carried out in a tea estate near Siliguri, Bengal Duars. The results were summarised earlier in this report.

Observations were also conducted on the bionomics of *A. minimus* with special reference to the time of biting. As the results of the night catches the largest number of freshly fed specimens were collected between 0300 and 0600 hours and the least number from 1800 to 2100 hours.

7. Statistical enquiry into the methods of forecasting epidemic malaria under Khan Bahadur Dr. M. Yacob, Assistant Director of Public Health, Punjab, and Mr. Satya Swaroop, Statistical Officer, Public Health Department, Punjab, Lahore.

The purpose of this enquiry is to eliminate the personal factor, and thus to place on an objective footing, the method which has so far been in use in the Punjab for forecasting epidemic malaria. The enquiry was in its second year.

The investigation on the periodicity of epidemic malaria was completed during 1945 and a paper embodying the main conclusions has been published in the Journal of the Malaria Institute of India in the June 1945 issue.

In the annual report for 1944 a brief mention was made of the work done to evaluate the degree of association between rainfall and malaria. This work was continued. The rainfall data were compiled on monthly basis from the original records in the office of the Director of Land-Records, Punjab. The annual epidemic figures for the province as well as for each district separately was correlated with the rainfall in each of the twelve months of the respective areas. Altogether 360 coefficients of correlation were thus worked out.

This analysis shows that, of all the months, rainfall in May, July and August was more closely correlated with the epidemic figure than in other months.

The interesting finding is that in quite a large number of districts rainfall in the month of May is correlated significantly with the malaria epidemic figure of the following autumn. This relation does not seem to have been suspected by previous workers. The precise manner in which the few showers of rain during the hot month of May give rise to malaria in the last quarter of each year remains yet to be determined. This result provides us with an altogether new factor for predicting malaria three months in advance. The calculations made, however, show that the degree of relationship of May rainfall with subsequent malaria varies considerably from district to district. In a few districts, viz., Hissar, Gurgaon, Montgomery and Lyallpur, it is the rainfall in April that is significantly correlated with malaria in the last quarter of each year. Considerable variation in respect of this association is found in regard to rainfall in other months also. For instance, even the August rainfall in the hilly districts

of Simla and Kangra bears no relation to the subsequent malaria epidemics. The results emphasise the point that each individual place requires a specifically designed regression equation for purposes of predicting malaria epidemics. The results of this study have been incorporated in a paper entitled " Rainfall and Malaria in the Punjab " which is being submitted to the Journal of the Malaria Institute of India.

A study has also been carried out to see whether the degree of relationship between rainfall and malaria epidemic figures during the last 77 years has undergone any change. It is of interest to report that correlation of the rainfall in May and August with malaria has increased markedly in recent years and that it is only in the current century that rainfall in May has begun to show statistically significant relationship with an epidemic of malaria in the autumn. It is presumably due to the introduction of canal irrigation and the development of waterlogging conditions associated with it in the Province that this change has been brought about. It is proposed to carry out this study further.

Preliminary investigation was also carried out to study the relationship between spleen indices and the subsequent malaria incidence. Gill, who devised the method of forecasting malaria, believed that spleen index was an important factor for predicting malaria. For the province as a whole no statistically significant relationship has been established. The cumulative effect of the spleen indices of two or more previous years on the intensity of subsequent malaria epidemics is under investigation.

In recent years some epidemiologists in the United Kingdom and the United States of America have suggested that variations in sunspot numbers may be related to epidemics of malaria and several reasons why such a relation may exist have been put forward. Gill also in his recent writings has expressed his belief in such a relationship. However, no convincing statistical proof for or against this contention seems yet to have been presented. The relevant literature on the subject has been studied and the basic data relating to 78 years collected. The statistical significance of the effect of spottedness of the sun on malaria is under investigation.

8. Mammalian malaria enquiry under the Director, Central Research Institute, Kasauli.

This enquiry was undertaken in response to a recommendation made by the Sub-Committee on Tropical Diseases of the Royal Society. The organisations which are collaborating in the work include the Royal Society, the Department of Health of the Government of India, General Headquarters of the Army in India, and the Indian Research Fund Association. The primary object of the enquiry is to determine whether or not an exo-erythrocytic cycle of parasite development occurs in mammalian malaria. The method of experimental study which has been selected is the infection of *M. rhesus* monkeys with *P. cynomolgi*. The work of the enquiry commenced on the 15th May 1945, but the histo-pathologist and the pathological technician did not arrive from the United Kingdom until the 20th September 1945.

The progress of the work in collecting material for histological examination was delayed due to the initial failure to transmit the infection by mosquitoes.

This failure was attributable to the high mortality which occurred among the mosquito population and which was caused by the use of D.D.T. in the neighbourhood of the work in Delhi. It became necessary, therefore, to transfer the entomological section of the enquiry to Karnal and make a fresh start. During September and October mosquito transmission was successfully carried out at Karnal and material collected for histological study.

Experimental mosquito transmission of *P. cynomolgi* to *M. rhesus* monkeys.

Adult mosquitoes reared from larvae were used for the transmission of *P. cynomolgi* to *M. rhesus* monkeys. The larvae were collected from breeding places in the field. The adult mosquitoes were fed on gametocyte carries which were obtained by infecting *M. rhesus* monkeys with parasitised blood by inoculation. During the period of parasite development the mosquitoes were stored in a room under ordinary temperature conditions and with a relative humidity maintained at about 80 per cent. Difficulty was experienced in obtaining large numbers of infected mosquitoes due to the high mortality among the mosquito population during the period of parasite development and due to the low index of experimental infection of the mosquitoes.

Preliminary observations indicate that the numerical prevalence of gametocytes is not the only factor concerned in determining whether or not mosquitoes will become infected after feeding. The results obtained so far suggest that mosquitoes fed on gametocyte carries early in the course of the primary attack when the gametocyte prevalence is low are more likely to become infected than mosquitoes fed at the peak of parasitaemia when the gametocyte prevalence is high.

The anopheline species which have been found suitable for the experimental transmission of *P. cynomolgi* to M. rhesus monkeys include *A. subpictus*, *A. culicifacies* and *A. annularis*. Successful transmission to M. rhesus monkeys has been obtained not only by the bite of infected mosquitoes but also by the intravenous inoculation of infected salivary glands dissected in serum-saline mixture.

Morbid anatomical and histological work.

The morbid anatomical and histological work has been in progress for two months and is still in its experimental stage. The problem is to find the most reliable and economical way of making large numbers of preparations and staining them in such a way as to show all forms of the malarial parasite and at the same time to be able to carry out detailed cytological differentiation. The most promising results have so far been obtained with a modification of the Romanovsky stain described by Barrett (1944, Jour. Path. & Bact., *56*: 133). Touch and smear preparations are also being used.

Other investigations.

Other investigations which are in progress include tissue transmission experiments, experiments to determine the fate of the sporozoite and studies to determine the natural course of *P. cynomolgi* infections following blood inoculation and sporozoite inoculation.

9. Enquiry on immunology in malaria under Dr. B. M. Das Gupta at the School of Tropical Medicine, Calcutta.

Preliminary experiments were made to find out (1) the immunological relationship between Plasmodium knowlesi and the different species of human malaria in human hosts and (2) the immunological relationship between the strains of human species from different localities. Five cases have been studied and the results are summarised below :

Case 1. B.T. infection induced by blood inoculation gave immunity to 2 subsequent inoculations of B.T. (one with infected blood and the other with sporozoites) from local and therefore probably homologous strains, given within a period of about 4 months after the disappearance of the primary infection.

Case 2. B.T. infection induced by sporozoite inoculation gave immunity to 5 subsequent inoculations of B.T. sporozoites given within a period of about 4 months. The first reinoculation was with the identical strain and the other reinoculations were with strains from different localities of Bengal.

Case 3. This was a case of chronic, very relapsing type of natural B.T. infection and was under observation for about 8 months during which the patient had 7 courses of anti-malarial treatment followed on each occasion by a relapse. He had 2 inoculations with infected blood containing P. knowlesi but they failed to produce infection. He was discharged as he was found unsuitable for further work in view of his absolute lack of immunity response.

Case 4. This case had 5 inoculations with infected blood containing different species of malaria parasites at intervals of a month or so, with the following results :

(1) Inoculation with P. knowlesi : infection terminated by spontaneous recovery.

(2) Inoculation with local strains of P. vivax : the resulting infection was terminated after 3 weeks with small doses of quinine.

(3) Inoculation with P. malariae : the resulting infection was terminated after 3 weeks with small doses of quinine.

(4) Inoculation with Burma strain of P. vivax : after an incubation period of 14 days both P. vivax (? Burma strain) and P. malariae appeared in the blood. The infections were terminated after sometime by small doses of quinine.

(5) Inoculation with local strain of P. vivax : infection after a slightly longer incubation period of 24 days.

Case 5. During the period of 6 months that the case was under observation he had 4 blood inoculations with different species of parasites and the results are as follows :

(1) Inoculation with P. knowlesi : infection terminated by spontaneous recovery.

(2) Inoculation with local strain of P. vivax : the resulting infection was allowed to continue for $3\frac{1}{2}$ months during which there were 2 mild relapses followed by spontaneous disappearance of parasites.

(3) Inoculation with P. knowlesi : no infection occurred.

(4) Inoculation with P. falciparum : infection resulted after normal incubation period. This was accompanied by a relapse of the B.T. infection. For reasons beyond our control, the case had to be discharged at this stage.

Summary : With the exception of case No. 3, which was of a persistently relapsing type, the immunity response in the rest of the cases may be summarised as follows :

(1) P. knowlesi infection confers no immunity against human species of Plasmodia ;.

(2) Local and other Bengal strains of B.T. are probably homologous ;

(3) In case No. 1, there was no evidence of antigenic difference between asexual forms and sporozoites of the local strains of P. vivax.

10. Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta.

The work carried out during the year 1945 may be summarised as follows :—

Filariasis control.

The feasibility of filariasis control was demonstrated at Ratanpur in the Central Provinces where endemic filariasis due to *Wuchereria malayi* infection has been previously reported (Ind. Jour. Med. Res. *28*, p. 609). The transmitter of the infection was found to be mansonioides. The control measure employed was the removal of Pistia from ponds, since in the absence of Pistia plants mansonioides is incapable of breeding. The work was carried out in collaboration with the Public Health Department of the Central Provinces. By the beginning of this year all the ponds had been cleared of Pistia and in consequence the incidence of mansonioides mosquitoes was brought down to a very low level. The control of mansonioides is being maintained and the work is now in the hands of the military department.

The effect of this measure would be a reduction in the transmission of malayi infection which could be demonstrated if this scheme is continued for a few years.

Filariasis survey.

Narkher, a village in Nagpur taluq (C.P.) is endemic for filariasis. Here the infection is due solely to *W. bancrofti*. Out of 57 persons examined for the infestation 17 were positive. The mosquito fauna consisted of *Culex fatigans* and *Armigeres obturbans*. No mansonioides were present in the area.

Katni, in Jubbulpore district (C.P.) is another focus of *W. bancrofti* infection. It is a growing town with a population of 30,000, a centre for lime and cement mines and lies on the main railway line from Calcutta to Bombay (E.I.Rly.). The infestation rate for Katni is 9 per cent (3 persons in 35 were positive). This town is another instance of sporadic infection in inland area like Bilaspur, Raipur and Nagpur in the C.P. all of them lying on the main line of communication (B.N. Rly.) and all the areas showing moderate filarial infection.

While on a visit to Cuttack in May 1945 opportunity was availed of to observe the incidence of filariasis in the town and also the incidence of culex breeding. It was found that the present condition was similar to what was observed during investigations carried out in 1922 and 1930.

Morphological studies of filarial parasites.

A detailed study was made of microfilariae collected from Ratanpur (*W. malayi* area) and from Narkher (*W. bancrofti* area) with regard to measurements and morphological characters and it was found that there was no marked variation from the normal.

Studies in pathology of filarial infection.

Investigations are in progress in order to find out whether any destruction of microfilariae occurs in the spleen tissue, as reported by Dhayagude and Amin in the Amer. Jour. Path. Vol. 18 p. 351. Of the 14 cases so far obtained from autopsy cases none of them was positive. This work is being continued.

Treatment of filarial infection.

Further investigations in the treatment of filarial infection were carried out with the following metallic compounds and vegetable drugs.

Anthiomaline.—Lithium antimony thiomalate, an organic trivalent compound of antimony containing 16 per cent of the metal made by Messrs. May & Baker, London, in 1 to 4 c.c. doses intramuscularly two or three times a week till a total of 20 injections is given.

This compound had been tried by the writer in 1937 and reported as beneficial in controlling the attacks of acute lymphangitis in cases of filarial infection vide Ind. Jour. Med. Res. *27*, p. 549. Recently a report on the usefulness of this compound having appeared in the Jour. Amer. Med. Assn. (125, p. 952, Aug. 5, 1944) it was used again in a number of cases of filarial infection and the above findings confirmed. The compound, however, has no lethal effect on the parasite.

Stibatin (*Glaxo*).—A pentavalent compound of antimony of low toxicity containing 20 mgms. of the metal per c.c. It is administered intramuscularly daily in doses of 10 c.c., 15 c.c. and 20 c.c., the last dose repeated for ten injections. This preparation is much less toxic than anthiomaline and is excreted more rapidly from the system. No lethal effect could be observed on the parasites after full course of treatment.

Mapharside (*Parke Davis & Co.*).—A well known compound of arsenic with 31 per cent of arsenic in trivalent form. Administered intravenously at weekly intervals beginning from 0·02 gm. to 0·06 gm., patients appear to respond well to this drug treatment although it is too soon to assess any value to this compound with regard to its efficacy in warding off recurring attacks of lymphangitis. In two cases of chyluria, however, the urine cleared up completely after 3 to 4 injections. The drug did not appear to have any lethal effect on the parasite.

Filarsen (*Polyclinic Lab.*).—Reported to be a combination of arsenic salt in solution with emulsions of killed T.A.B. and strepto-coccal organisms. It is claimed that this compound is superior to Arseno-typhoid, a combination of arsenic compound with T.A.B. organisms only. The latter preparation is widely

used and reported to be effective in controlling recurring attacks of lymphangitis in filariasis. In a series of 58 patients treated with Filarsen, it was observed that although the compound showed no effect on the parasite, it appeared to improve the general tone of the patients thus helping them to overcome the susceptibility to attacks of lymphangitis.

Mist Calotropis Co.—A preparation chiefly of *Calotropis gigantea* administered orally. Although the manufacturers claimed anti-filarial properties to this vegetable extract it did not show any such effect as are claimed for it when tried in a series of patients both in the hospital and in the outpatients department.

Penicillin.—While penicillin is lethal to microfilariae in vitro, no such effect was observed in the human system. No beneficial effects were observed in cases of chyluria treated with adequate doses of penicillin.

NUTRITION

11. Nutrition researches under Dr. W. R. Aykroyd at the I. R. F. A. Nutrition Research Laboratories, Coonoor.

Work carried out in the Nutrition Research Laboratories during the year has included the following :—

(1) The analysis of foodstuffs.

1. The thiamin and nicotinic acid content of different samples of yeast, yeast extracts, wheat, rice, vitamin tablets and ampoules and prosopis has been determined.

2. Further work has been done on the antithiamin factor in foodstuffs. It was observed that substances such as mercuric chloride and silver nitrate interfered with the estimation of thiamin by the thiochrome method and it was felt to be of interest to discover whether other substances will behave in a similar way. Bromine, iodine and sodium pyrophosphate were found to exert a similar interfering effect.

3. The nutritive value of prosopis was investigated at the request of the Punjab Government with a view to finding out if it could be used as a human food during times of food emergency. Its chemical composition did not reveal anything striking, but feeding trials showed it to be highly toxic to rats (young and adult) when consumed in the raw and in the cooked state.

4. Work on the estimation of para-amino-benzoic acid and thiamin by a simple colorimetric method has just been started.

5. The report on soya bean was completed and sent to the office of the I.R.F.A., for publication.

6. A modification of Scudi's colorimetric method of estimation of pyridoxine is being worked out. Attempts are also being made to apply this method to the estimation of pyridoxine in biological materials.

7. The fractionation of the nitrogen and phosphorus complex of the coconut kernel has been completed. The carbohydrate complex of the kernel is being studied.

8. Experiments are in progress on the loss in vitamin A content of ghee when stored under different conditions.

(2) Animal experiments.

1. Previous observations on the acceleration of growth of guinea-pigs by hesperidin (vit. P) have been confirmed. Attempts are now being made to develop a method for determining the minimum amount of hesperidin required for growth and maintenance of guinea-pigs.

2. Experiments on lathyrism using guinea pig as the test animal are being carried out.

3. Experiments on rat leprosy were continued.

4. Biochemical changes occurring in the tissues of rats suffering from thiamin deficiency have been completed. A similar study is being made on rats kept on a riboflavin deficient diet.

5. Studies on the excretion of nicotinic acid excreted by young and adult rats given this vitamin at different levels of intake were continued.

6. Consumption of salt-rich diets is stated to aggravate symptoms of vitamin C deficiency, presumably through changes in the intestinal flora. Experiments were therefore carried out with rats employing salt-rich diets either alone or in association with a bacteriostatic agent. While 5 per cent sodium chloride in the diet led to depraved appetite and consequent loss in weight of the animals, 1 per cent sodium chloride either alone or along with sulfasuxidine had no deleterious effect. Symptoms of vitamin C deficiency were not observed with even 5 per cent Nacl.

7. Studies on the metabolism of choline were continued. It was found that growth measurements do not indicate the extent of fatty degeneration of liver. Phosphorus assimilation was found to be adversely affected in cases of choline deficiency.

8. The supplementary effect of the proteins of food yeast, casein, skimmed milk powder, egg and groundnut on the proteins of rice is being studied both by the growth method and the balance sheet method.

9. Experiments on the comparative nutritive value of animal and vegetable fat have been carried out. Some evidence has been obtained indicating that the animal fats, ghee and lard, give better increase of growth per gram of fat than vegetable oils.

10. Investigations on the comparative availability of calcium from such salts as calcium carbonate, calcium gluconate, calcium lactate, calcium hydroxide and calcium phosphate are now in progress.

11. Preliminary experiments were carried out to study the changes in the blood in fluorine poisoning in rats.

12. Investigations on the nutritional properties of the coconut kernel were commenced.

(3) Clinical and field investigations.

(a) The work on infantile beriberi in Cocanada has proceeded and a report on this enquiry is under preparation. While the curative effect of pure vitamin B_1 on infants suffering from beriberi has been abundantly confirmed, a preventive experiment in which vitamin B_1 tablets supplying one milligram of vitamin B_1 were given daily to expectant and nursing mothers was not wholly successful. Infantile beriberi developed in some of the babies born to these mothers. It appears that the amount of the vitamin given was not sufficient for completely satisfactory prevention.

During the last 2 months however the progress was not upto expectations owing to the death of the lady doctor who was in charge of the centre in Cocanada. The enquiry will be closed on 30-11-1945 as it has not been possible to secure the services of a suitable lady doctor.

(b) An investigation on lathyrism in Bhopal State was carried out in February 1945. A paper has been prepared on this subject for submission to I.J.M.R., and the attention of Food Department has been drawn to certain aspects of the outbreak in question, and in particular to the fact that wheat procurement operations played a part in the genesis of the epidemic. The outbreak was due to the consumption of lathyrus as a staple food for six months

or more, and no confirmation was obtained of the theory that a poison in the pulses *vicia sativa* may be concerned in the causation of lathyrism.

(c) Work on the relation between dental caries, fluorosis and nutrition was continued.

(4) ADVISORY WORK.

Advisory work continued as usual, requests for information and advice being received from many parts of the country.

(5) NUTRITION CLASS.

A course of lectures on nutrition were delivered to 26 officers selected by the Department of Food, Government of India from various government departments of Provinces and centre, from 1st to 30th July 1945.

(6) From the end of April the Famine Inquiry Commission was housed in the Nutrition Research Laboratories in the Pasteur Institute and made use of the library and museum.

12. Ascorbic acid enquiry under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

ON THE MECHANISM OF THE BIO-SYNTHESIS OF ASCORBIC ACID.

It had been previously shown that narcotics can accelerate the synthesis of ascorbic acid in rats without affecting significantly urinary sugar, nitrogen and phosphorus, blood sugar and phosphorus and liver glycogen. It had been further observed that high ascorbic acid excretion was not due to any glucogenic residue of " extra protein " than that required for normal catabolism, nor had it resulted from any disturbance in phosphorylation. It had, moreover, no apparent connection with the detoxicating mechanism of the rat or the failure of any component in it. Injection of intermediary metabolites, such as pyruvate, lactate etc., had no effect on the synthesis. The process was also found to be independent of the endocrine factors, as the excretion under narcotics remained unaffected by adrenalectomy or pancreatectomy of the rats. Amongst other dehydrogenase systems studied, pyruvic and lactic seemed to be affected in narcotised rats. It was then observed that the accelerating influence of narcotics on the biosynthesis of ascorbic acid by rats could be almost completely suppressed, when the rats were made vitamin B_1 – deficient. About 100 cases have so far been studied with the same result. After the rats were rendered B_1-deficient, they were given orally the usual dose of narcotic daily. The urinary ascorbic acid was determined chemically for a period ranging between 1-2 weeks. The excretion values were always very low : traces or in some cases 3-6 mg.%, as compared to 30-100 mg.%, the values for normal narcotised rats for the same period. The urine of B_1-deficient narcotised rats was also tested by the biological method of assay and was found to contain practically no ascorbic acid. When these rats were subjected to B_1-therapy (0·25-0·5 mg. daily, injected or oral) it was observed that urinary excretion started after 3-4 days, gradually attained and kept to the usual level (30-100 mg.%) within 2-3 weeks. This was also corroborated biologically.

Control rats maintained on B_1-deficient diet, autoclaved yeast etc. but supplemented with 0·25-0·5 mg. B_1 daily, began to excrete ascorbic acid when attained and kept to the usual level (30-100 mg.%) within a week (in some cases even earlier). Some of those control rats were subjected to 24-48 hours' fast for a study of the inanition effect. It was, however, observed that inanition has no effect on the usual excretion of ascorbic acid (30-100 mg.%) by rats under narcotics.

It was also thought desirable to investigate whether any extra ascorbic acid is needed or destroyed in rats due to their abnormal state during B_1-deficiency. Heavy doses of synthetic ascorbic acid (50 mg.) were therefore injected into B_1-deficient rats without narcotics and the recovery of ascorbic acid in the following 24-hours' urine was 15-20 mg. which agreed fairly well with the values for normal controls, injected with the same dose of the vitamin (50 mg.).

It has been previously observed by others that fat has some sparing action on thiamine. 10% of the total food intake by B_1-deficient and narcotised-B_1-deficient rats was therefore substituted by butter and the urinary excretion of ascorbic acid under both the conditions determined for a period of 1-2 weeks, which was found to be within the usual low limits (traces—5 or 6 mg.%).

Even considering the effect of inanition, the sparing action of fat, and the possibility of ascorbic acid being destroyed in vivo during the abnormal state in B_1-deficiency, it is apparent that vitamin B_1 is involved in the biosynthesis of ascorbic acid by rats, at least as it is accelerated by narcotics.

Effect of depletion of one factor on the other.

Since this interdependence between the vitamins B_1 and C had been observed, it was of some interest to see whether with depletion of B_1 there was corresponding diminution of C or vice versa in different tissues, such as, brain, liver, kidney and blood of rats and guineapigs. It was found that the ascorbic acid level in the above tissues of rats, normal or B_1-deficient, remained practically the same.

The B_1 level determined according to Harris *et al.* (Biochem. J. 1941, *35*, 1050) in different tissues of guineapigs, normal or scorbutic also showed no significant difference.

Possible relationship with "Citric Acid Cycle".

It has been observed (Elvehjem *et al.* J. Biol. Chem. 1940, *134*, 605) that there is a fall in the citric acid excretion by rats during acute thiamine deficiency, which is restored to normal values within 10-14 days by B_1-therapy. Now by B_1-therapy ascorbic acid excretion by avitaminous rats under narcotics is also restored to normal values within this period. It was, therefore, of some interest to see whether ascorbic acid synthesis and its acceleration under narcotic conditions were in any way dependent on or independent of the citric acid process or the so-called "citric acid cycle" of Krebs. Urinary excretion of citric acid by rats under various conditions was determined according to Pucher *et al.* (J. Biol. Chem. 1936, *113*, 235). It was found that citric acid excretion was not hampered by narcotics during B_1 therapy but ran parallel with the rise of ascorbic acid. It appears, therefore, that ascorbic acid synthesis restored in avitaminous rats under narcotics by B_1-therapy is independent of the citric acid process.

FATE OF PYRUVIC ACID IN B_1-DEFICIENCY AND UNDER NARCOTICS.

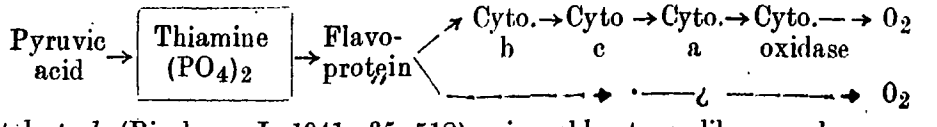
During B_1 deficiency there is a considerable accumulation of pyruvic acid in the blood of avitaminous animals (Peters and co-workers). It seemed, therefore, of interest to see whether high blood pyruvic acid level of avitaminous rats was in any way affected by narcotics. Blood pyruvic acid of rats in normal and avitaminous conditions was determined according to Lu (Biochem. J. 1939, *33*, 774) with certain modifications by Long (*Ibid*, 1942, *36*, 810) and by ourselves.

It was observed that narcotics had no effect on the blood pyruvic acid accumulated during B_1 deficiency.

ACTION OF INHIBITORS.

During B_1-deficiency pyruvic acid oxidation is greatly inhibited and disturbed. Attempts were, therefore, made to produce *in vivo* certain disturbances of pyruvic acid oxidation by means of some inhibitors, such as iodoacetate, malonate, fluoride etc. These inhibitors separately or in combination of 2 in carefully adjusted doses were injected daily into normal rats along with narcotics. The ascorbic acid excretion under narcotics was not, however, affected by these inhibitors but attained the usual high level within the usual period.

It appears from the above findings that thiamine plays a very important role where biosynthesis of ascorbic acid by rat is accelerated under narcotics. Now, thiamine or its phosphorylated form diphosphothiamine (co-carboxylase) is closely associated with pyruvic acid metabolism in the living organisms, including plants. Barron *et al.* (J. Biol. Chem. 1941, *141*, 957) have shown that diphosphothiamine acts by forming an integral part of the activating protein of the enzyme systems concerned with the activation of pyruvate. Therefore, diphosphothiamine is indispensable for and inseparably connected with pyruvic acid metabolism. Our experimental evidences clearly indicate that thiamine or better diphosphothiamine (since thiamine acts after enzymatic formation of diphosphothiamine) is indispensable for ascorbic acid synthesis by rats. So it is quite conceivable to regard ascorbic acid synthesis as a phenomenon of pyruvic acid metabolism. Ball (Cold Spring Harbor Symposia Quant. Biol. 1939, *7*,100) has presented evidence for a scheme of pyruvic acid metabolism in living organism as follows :—



Quastel *et al.* (Biochem. J. 1941, *35*, 518) using chloretone, like ourselves, as narcotic and with rat and other mammalion tissues, have presented strong evidence that the site of action of narcotics involved a special flavoprotein, suggesting cytochrome C reductase of Haas (J. Biol. Chem. 1940, *136*, 747) as a probability. The failure of narcotics in accelerating the biosynthesis of ascorbic acid by rats under B_1-deficiency, as observed by us, can be very appropriately explained from the above scheme. This scheme, also, seems to suggest something of a precursor role to the pyruvic acid for the synthesis of ascorbic acid. In view of the central position that pyruvic acid occupies and the manifold reactions it is capable of undergoing in the metabolic process of the living organisms, such a precursor role for this keto-acid seems not an

improbability. This scheme, as the probable path of formation of ascorbic acid, also, suggests some important role for the flavo-proteins. By knocking out the flavin component of the chain of reactions involved in this scheme, there is also some likelihood of the failure of synthesis of ascorbic acid. Some experimental evidence has already been obtained which seems to support this view.

13. Enquiry on the formation, function and variation of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

The importance of plasma proteins in maintaining the fluid balance of the body was shown by Starling in 1896. Clinical observations were stimulated by the work of Peters in 1926. The significance of his studies was overlooked for many years. It is only recently that plasma proteins are receiving considerable clinical importance. Most of the work on the subject is based on the determination of albumin and globulin only and covers small number of cases. In this country very little work has been done on the subject.

The results of fractionation of proteins from the blood of patients from a large varieties of diseases reveal that the data falls naturally into two main groups :—

(1) Cases in which the total protein is distinctly below the normal level (hypoproteinemia).

(2) Cases in which the total protein is either normal or above normal (hyperproteinemia).

In view of the considerable increase in the number of cases that are admitted to the hospital with oedema and ascites, the investigation was restricted to the study of cases with *hypoproteinemia.*

Hypoproteinemia. Hypoproteinemia is undoubtedly due to a lowering of albumin, but still the cases can be studied under two heads.

(1) The group of cases in which the concentration of albumin is lowered and the globulin remains within normal range.

(2) Cases in which the values of globulin are slightly higher than the normal, but the total protein is below normal due to greater decrease in the concentration of albumin.

The first group includes anaemia, osteomalacia and malnutrition due to inadequate intake of protein or inadequate absorption. The second group includes various liver diseases, such as cirrhosis, nephritis, etc.

The clinical significance of plasma proteins—the relationship of albumin, globulin and the total protein.

Determination of plasma proteins are of considerable clinical significance. An accurate determination of different protein fractions of the blood plasma is laborious and time consuming. From a clinician's point of view it is important to know whether the value of total protein is sufficient,—if so, in what conditions. It is also important to know the types of cases in which an accurate knowledge of albumin and globulin is informative.

The result of present series of studies clearly indicate that the value of total protein alone is of no clinical significance. But, there are types of cases in which the values of albumin and globulin can be accurately calculated from the value of total protein, by the application of a certain formula which is based on a large number of cases in each series and the results are shown below. Different formulas have been worked out for anaemia, nutritional oedema, acute and subacute-nephritis.

Anaemia. In anaemia, the concentration of total protein varies widely; with a range from 3·5 gram to 7·5 gram %. The globulin is either normal or slightly below normal. In only 4 out of 50 cases was the globulin above normal due undoubtedly to associated infection. The variation in the value of total protein is due to variation in the value of albumin, so that a linear relationship exists between the total protein and albumin. The value of albumin can therefore be calculated from the value of total protein by the application of the following equation :—

$$(\text{Total protein} - 2{\cdot}0) \times 0{\cdot}9 = \text{Albumin}.$$

Hence the value of globulin can also be determined accurately.

TABLE 1.

The observed and the calculated values of albumin from total protein in anaemia.

Total protein Gram %	Observed value of albumin Gram%	Calculated values of albumin Gram%
7·12	4·51	4·60
6·56	4·00	4·10
6·20	3·89	3·78
6·25	3·75	3·82
6·11	4·01	3·70
6·06	3·56	3·65
5·86	3·66	3·47
5·85	3·65	3·46
5·85	3·40	3·46
5·50	3·15	3·15
5·24	2·98	2·91
4·94	2·71	2·64
4·91	2·58	2·61
4·75	2·59	2·47
4·62	2·34	2·35
4·06	1·75	1·85
4·02	1·92	1·81
3·75	1·53	1·57
3·56	1·25	1·40

From the results of table I, it is evident there is absolute agreement between the observed and the calculated values of albumin.

A large number of cases of anaemia associated with oedema and ascites are now being admitted to the hospital. For correction of hypoproteinemia and oedema in these cases it is essential to know the value of albumin and

globulin. Determination of total protein alone will suffice in these conditions since, the values of albumin and globulin can be calculated from the total protein with sufficient accuracy for clinical purposes.

Nutritional oedema. A characteristic oedema appears widespread during war time. This war was no exception. Raynaud (1943) found an average value of 5·39 gram% of total protein in some persons with this oedema. In the present series, 30 determinations on 19 cases shows that the total protein was below 5 gram% in 20 and between 5 to 6 gram in the remaining 10 determinations.

In cases of nutritional deficiency state, the value of globulin tends to fall towards the higher range of normal. The value of albumin can be calculated from the total protein by the application of equation—

(Total protein—2) ×0·8=Albumin.

TABLE 2.

Observed and calculated values of albumin in nutritional deficiency.

Total Protein	Observed value	Calculated value
	Albumin (gram%)	
6·25	3·38	3·40
5·48	2·86	2·78
5·31	2·46	2·64
5·12	2·71	2·49
4·51	2·40	2·00
4·42	1·97	1·93
4·16	1·70	1·72
4·06	1·76	1·68
4·25	1·77	1·80

In cases of nutritional deficiency states the values of albumin can be calculated from the value of total protein with sufficient accuracy for clinical purposes.

The cases of nutritional oedema may be classified into 3 groups (1) Those responding to high protein diet. (2) Those who respond only when vitamin B_1 is supplemented with high protein diet. (3) Those which do not easily respond to such treatment. It is in this group that one finds an appreciable difference between the observed and calculated values of albumin. This may be due to associated infection or due to the fact that prolonged malnutrition might have resulted in a damage of the liver. This group deserves further investigation.

Nephritis. In all cases of nephritis, the globulin is above normal but usually below 3·5 gram%. In most of the cases of subacute nephritis, the total protein is below 5 gram %, in spite of the increase in the value of globulin. The value of albumin can be predicted fairly accurately from that of total protein from the equation :—

Subacute nephritis :— (Total protein—2)×0·6=Albumin.

In acute nephritis the albumin is at a much higher level and so also the total protein. The value of albumin can be calculated as follows.

Acute nephritis.

(Total protein—2)×0·75=Albumin.

TABLE 3.

Observed and calculated values of albumin in subacute and acute nephritis.

Total Protein gram%	Albumin gram%		Nature of disease
	Observed	Calculated	
4·74	1·74	1·64	Subacute
4·94	1·72	1·76	
5·12	1·89	1·87	
4·81	1·98	1·68	
4·43	1·32	1·45	
4·93	1·70	1·75	
4·21	1·47	1·32	
4·73	1·30	1·63	
5·62	2·14	2·17	
3·75	1·24	1·05	
7·68	4·24	4·31	Acute nephritis
7·93	4·36	4·44	
8·81	5·25	5·10	
6·43	3·62	3·36	
6·06	3·09	3·04	
5·90	2·95	2·92	

In cases of nephritis the total protein value is sufficient to predict the value of albumin and globulin.

Cirrhosis. In cases of cirrhosis, the albumin and globulin vary so widely that it is not possible to predict the value of either of these constituents from those of total protein. Hence the value of albumin and globulin should be determined separately.

Total protein	Albumin		Globulin	euglobulin	pseudoglobulin
7·62	4·31		3·31	0·91	2·40
7·31	3·66	"	3·65	1·64	1·80
7·25	3·29		3·96	0·88	2·86
7·57	2·85		4·72	2·02	2·47
7·81	2·32		5·52	3·12	2·17
9·06	2·86		6·20	2·75	3·25

The decrease in the value of albumin and increase in globulin, possibly indicate the course and stage of the disease. As globulin increases, there is increase in euglobulin which is significant of this disease. Further work is in progress.

In cases of liver diseases and tuberculosis, it is essential to determine the values of albumin and globulin accurately since both constituents vary widely.

14. Enquiry on protein metabolism by human feeding experiments under Dr. S. Ahmad at the Public Health Laboratories, Bankipur, Patna.

STUDIES OF PROTEIN METABOLISM WITH INTERCHANGE OF CEREALS IN THE RICE DIET.

Introduction.

Scarcity of food grains during 1943-44 had brought into prominence the question of interchangeability of cereals in the diet and the introduction of less familiar cereals in certain parts of India. In the present investigation an attempt has been made to find out whether partial replacement of wheat flour in the chapati by an equivalent weight of flour from maize and the different millets effect any appreciable difference in the biological value of the digestibility coefficient.

Rice diet.

The feeding experiments were conducted on six adult human males weighing from 40 to 58 kg. The test diet contained medium quality home-pounded parboiled rice 550 g. in the case of the first two subjects, 800 g. in the next two subjects, and 650 g. in the case of the last batch of two subjects. The respective figures for the pulse (Red gram, cajanus Indicus) was 75 g., 110 g. and 90 g. The diet was supplemented by 120 g. of potato, 150 g. of bottle gourd and 200 g. of leafy vegetables cooked in vegetable oils. Total amount of protein in all the diets was almost constant for each subject. Every subject was on this diet for a period of eight days and the faeces and the urine were collected during the last three days. The level of the protein intake was about 10%. The calorie value of the diet varied from 3000 to 3600 daily. The subjects performed normal duties during the test periods.

Mixed diet, rice and wheat.

The above feeding experiments were repeated on the same subjects. Half the rice quota was replaced by flour made from wheat, other things being constant, so that the first two subjects got 275 g. of rice and 234 g. of wheat flour. The respective figure for the next two subjects was 400 and 300 and for the last two subjects was 325 and 225. The other conditions of the experiment were identical with the previous experiment.

Mixed diet; rice, wheat and other cereals.

The above feeding experiments were repeated on the same subjects and in the same way except that 25% by weight of the wheat flour was replaced by flour made from barley, maize, junera, marua and bajra respectively in different periods. In these series of experiments the weight of the mixed flour was 240 g. in the case of first two subjects, 320 g. in the case of the other two subjects and 272 in the case of last two subjects. Other figures were the same as in previous experiments.

Biological values.

The average biological values of each of the test diets are enumerated below :—

Diet	Biological value
Pure rice diet (Oryza sativa)	66·6
Diet containing rice and wheat (Triticum vulgare)	55·1

Diet	Biological value
Diet containing rice, wheat and barley (Hordum vulgare) ..	59·8
Diet containing rice, wheat and maize (Zea mays)	56·8
Diet containing rice, wheat and junera (Sorghum vulgare) ..	54·1
Diet containing rice, wheat and marua (Eleusine coracana) ..	60·2
Diet containing rice, wheat and bajra (Pennisetum typhoideum) ..	57·4

DIGESTIBILITY COEFFICIENTS.

The mean digestibility coefficient on each of the test diets enumerated varied from 75 to 88. No significant difference between one diet and another could be detected.

15. Nutrition research unit under Dr. K. P. Basu at Dacca University, Dacca.

METABOLISM OF FAT.

Effect of a low fat diet on blood lipids : Numerous diet surveys undertaken in different parts of this country have shown that even in normal times the average daily fat intake per capita is very low, barely exceeding 1 oz., very little of which is of animal origin. The strain of war brought down this level of fat intake to a limit even lower than this and fats of animal origin (e.g., butter fat usually used) was beyond the means of the general population. The aim was to determine the effect on a healthy man of a diet practically devoid of fat. Uptill now investigations have been confined to the changes in the blood lipid level only.

Experiments were conducted on two normal healthy adult human subjects, who were kept on an ordinary vegetarian diet (rice, pulse and vegetables) without fat and with liberal supplements of lemon juice and sugar. This diet contained about 2 gms. of fat derived mainly from rice and vegetables. The experiment could only be continued for 2 weeks, but before this period the subjects were also on a low-fat diet (10-12 gms. per day) for nearly six months. The blood-lipid changes were determined at the end of this time. At the expiry of this period, the subjects were allowed to consume the same diet supplemented with 50 gms. of mustard oil and after one month, the blood-lipid changes were again noted. Results are given below :

Subject.	Condition of Expt.	Total lipids mg. p.c.	Cholesterol mg. p.c.	Iodine value of blood fat.
M.C.N.	Normal	608	239	98
(19 yrs.)	Subject on low-fat diet	552	226·8	83
M.C.D.	Normal ..	583	170	101
(18 yrs.)	Subject on low-fat diet	555	168	97

Side by side with the above experiment blood lipid changes were also observed in rats on the fat-free and normal diets. Following are the results,

Rat No.	Initial wt.	Final wt.	Condition of the Expt.	Total lipid mg. p.c.	Cholesterol mg. p.c.
1	175		Normal	354	75
		155	On fat-free diet	339	74
2	167		Normal	372	82
		151·5	On fat-free diet	365	85
3	153		Normal	383	81
		143	On fat-free diet	367	79

Vitamin B-complex.

(1) *Nicotinic acid:* Investigations were taken up to find out if any relationship existed between this vitamin and the metabolism of fat, protein and carbohydrate. The effect of administration of nicotinic acid on the glucose tolerance of rats was first determined. For this purpose 1, 2 and 3 mgs. of nicotinic acid were injected through the tail vein of rats and estimation of blood sugar was undertaken immediately before the administration of nicotinic acid and after intervals of 15 mins., 30 mins., 1 hr., 1½ hrs. and 2 hrs. The interesting observation was made that 15 mins. after the administration of nicotinic acid, the blood sugar level rose from 110 mgs. (average value) to 138 mgs. per 100 c.c. of blood. It then gradually decreased to normal after the lapse of 1½ hrs. or 2 hrs. depending on the magnitude of the dose of nicotinic acid administered. The greater the amount of nicotinic acid injected, the longer was the period of high blood sugar. This is thought to be a very important finding and it is proposed that various metabolic products of carbohydrate produced in the system on the administration of nicotinic acid should be estimated.

The estimation of nicotinic acid in various Indian foodstuffs is also being incidentally carried out.

(2) *Vitamin B_1:* The vitamin B_1 destroying power ("The chastek paralysis" factor) of some fresh water Bengal fish has been investigated. In most cases the vit. B_1 destroying power seems to be practically nil—with the exception of Shole (Ophcephalus striatus) and Bhetki (Lates calcifer). In this respect Shole is more active than carp. One gram of the fish-viscera destroyed 20 mg. B_1 in 2 hrs. Vit. B_1 was not destroyed by any sample of boiled fish.

The destruction of vit. B_1 of common Indian vegetables during cooking has been studied. All the vegetables investigated lost some of their vit. B_1 content during cooking. The average percentage retention of vit. B_1 in the case of 35 common vegetables after cooking is 59.

Investigation on the destruction of vit-B_1 during cooking has so far revealed that only the "free vit. B_1" is destroyed during cooking while the bound or the cocarboxylase content of the foodstuff remains unaffected. Further work is necessary to elucidate this point.

Soya bean.

(1) *Supplementing effect of soya-bean milk and cow-milk:* Growth experiments for investigating the supplementary effect of soya-bean milk and

cow-milk on the poor rice diet have been performed on a series of young rats weighing 50-60 gms. The average composition of cow-milk and soya-milk used was as follows :

	Sp. gr.	Total solid %	Fat %	Protein%	Ash %
Cow-milk	1·026	9·13	2·85	3·42	·67
Soya-bean milk	1·031	10·02	3·92	3·78	·43

The average increase in body-weight of rats per week is as follows :

Diet.	Average increase in wt. per week.
Poor rice diet without any supplement ..	3·44 gm.
Poor rice diet with cow-milk (10 c.c. per day) ..	8·65 ,,
Poor rice diet with soya-bean milk (10 c.c. per day)	7·25 ,,

(2) Experiments on the biological value by the growth method of some of the latest varieties of soya-bean and pulses have been completed. The results are reported below.

Sample.	$\frac{\text{Gain in body-wt. (gms.)}}{\text{Protein intake (gms.)}}$
Lyallpur soyabean	.. 0·88
Kashmir soyabean	.. 0·97
Bengal soyabean	.. 0·86
Manchurian soyabean ..	Sample deteriorated and all the rats died.
Bengal gram	.. 1·07
Dal arhar	.. 0·61
Green gram	.. 0·76

Food yeast.

Biological value of the proteins of food yeast supplied by the Department of Food, Government of India has been determined. On an average the food yeast was found to contain 51·75% protein, 8·5% moisture and 7·4 % ash.

(1) *Biological value of yeast by the growth method :* Experiment on the biological value of yeast by the growth method was performed on a group of 12 rats, and the ratio

$$\frac{\text{Gain in body-wt. (gms.)}}{\text{Protein intake (gms.)}}$$

was found on an average to be 1·19, while the same ratio for the best variety of soyabean so far investigated and for Bengal gram was found to be 0·97 and 1·07 respectively.

(2) *Biological value of yeast by the balance sheet method using rats :* The biological value of yeast by the balance sheet method was found on an average to be 71 and the corresponding digestibility to be 88 at 5% level of protein intake.

(3) *Supplementing effect of yeast on a poor rice diet:* The average increase in body-weight of rats per week was as follows:

Diet.	Average increase in body-wt. per week (gm.)
Poor rice diet without any supplement	3·44
Poor rice diet with 13% yeast	9·2

(4) *Biological value of food yeast proteins by a metabolism experiment on human subject.*—Experiments on the biological value of yeast were conducted on two human subjects—(N.C.D.) and (A.K.M.). The average biological value was found to be 72·2, and the corresponding digestibility to be 90·5 at 5% level of protein intake. Human subjects were also kept on an ordinary diet of known nitrogen content to which yeast was added. The N-excretion was followed and better retention was observed.

Mineral metabolism.

(1) The effect of Na-free, K-deficient, excess-Na and excess-K diets on carbohydrate metabolism as manifested by glucose tolerance and glycogen deposition in muscle and liver, has been studied in albino rats.

Rats on K-deficient and Na-free diets lost body weight and if the diets were continued for three weeks both classes of rats suffered from a disturbed carbohydrate utilisation as was evidenced by high fasting blood sugar level, loss in glucose tolerance and decreased glycogen deposition in liver and muscle. The effect of Na-deficiency on carbohydrate metabolism was, however, more marked than potassium deficiency.

Serum-Na and K-concentrations, too, underwent variations. The change in the concentrations of these two elements was of the complementary type. When the concentration of either element increased that of the other showed a fall. On a K-free diet serum K-concentration was depressed and Na-concentration was elevated and just opposite was the case when the rats were placed on a Na-free diet.

Excess-Na intake did not result in a noteworthy increase in the rate of growth. Improved glucose tolerance and increased glycogen deposition in liver and muscle were the remarkable features of the rats on excess-Na diet. Excess-Na intake also resulted in an elevated serum-Na level and a depressed K-level.

Excess-K intake exerted untoward effects both on growth and carbohydrate utilisation. The rats on excess-K diet lost weight and the loss in their glucose tolerance was most marked. Glycogen deposition too, was equally affected. Serum-K recorded a slightly elevated level while that of Na was slightly depressed, on excess-K intake.

Thus the effects exerted by Na and K were antagonistic in nature.

(2) The investigation of Indian foodstuffs as a source of available iron is being continued.

Effect of certain vitamins on cellular respiration.

Investigations have been carried out on the effect of vitamin B_1, A and D deficiency and also of an excess of vitamin A administration on aerobic and anaerobic oxidation in heart, liver, kidney and brain tissues of albino rats. The

substrates investigated were succinate, lactate and glycerophosphate. The oxidation of citrate was studied only in the anaerobic way.

The deficiency of vitamin B_1 led to the diminished aerobic oxidation in all the tissues in presence of succinate and glycerophosphate. But in the presence of lactate as substrate the B_1 avitaminous brain tissue respired practically normally while in all other tissues marked inhibition was observed. In cases of vitamin A and D deficiency all the tissues showed a considerably diminished respiration in presence of oxygen with all the metabolites investigated. The tissues from rats receiving excess of vitamin A exhibited peculiar phenomena. In this case the brain and liver tissues respired normally while kidney and heart tissues showed appreciably decreased respiration in presence of oxygen with all the substrates mentioned.

The anaerobic activity of the tissues diminished by a fairly large percentage in the case of B_1 deficiency. But in A and D avitaminosis all the tissues except brain showed a very great increase in activity. The activity of brain tissues, however, showed diminution with succinate and lactate as substrates while with glycerophosphate and citrate marked acceleration in activity was observed.

The tissues from rats receiving excess of vitamin A exhibited similar behaviour as in the aerobic process i.e. the activity of the liver and brain tissues was normal in presence of all the metabolites while that of heart and kidney tissues showed diminution.

16. Nutrition research unit under Professor S. P. Niyogi and Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

1. Fat metabolism.

(a) *Growth tests* :—The growth promoting properties of groundnut (*Arachis hypogea*) oil, and its partially hydrogenated product "*Vanaspati*" were compared with that of butter using albino rats as the experimental animals. The rats were fed *ad lib*, the food consumption and weekly increase in weight were recorded for eight weeks. In Table I are given the results of the experiment.

Table I.

Growth promoting property of some edible oils.

Diet at 5 per cent level of	No. of animals	Average daily food intake	Average gain in wt. per week per rat
		g.	g.
Butter fat	16	7·8	12·43
Groundnut oil ..	16	7·5	11·03
Vanaspati	16	7·5	10·85
Fat poor diet (control)	16	7·1	7·82

(b) *Metabolism experiments* :—Various fats at the intake level of 25 per cent of the diet were fed to six adult rats kept in metabolic cages. The urine and faeces were collected during the last four days of the eight day period. The analytical results are given in table II.

TABLE II.

The average results of metabolism experiments at 25 per cent level of intake of edible fats.

Diet at 25 per cent level of	Daily food intake g.	Daily fat intake mg.	Total lipoids in faeces mg.	Daily nitrogen intake mg.	Daily excretion in urine: Total N mg.	Daily excretion in urine: Ammonia N mg.	Total faecal *lipoids* × 100 Total fat intake	Total N in *urine* × 100 Nitrogen intake
Coconut oil ..	5·36	1340	108·8	200·6	95·7	6·6	8·12	47·71
Vanaspati ..	6·32	1570	149·2	236·6	94·5	7·7	9·5	39·94
Sesame oil ..	5·71	1430	142·8	213·8	90·9	6·4	9·99	42·52
Groundnut oil ..	5·2	1300	121·3	194·7	83·3	7·6	9·3	42·78
Butter fat ..	7·37	1841	59·4	277·0	131	9·7	3·2	47·2

The results show that at the level of intake tried in these experiments the residual faecal lipoids were slightly larger with vegetable oils than in the case of butter fat.

(c) *Absorption from the human intestine*:—Five subjects volunteered themselves for these experiments. They came to the laboratory in the morning with empty stomach. A sample of blood was taken by finger puncture and the standard test meal, consisting of 1 oz. of fat with 2 oz. of bread with a cup of weak tea, was given. Blood was obtained from the finger at fixed intervals. The sample was drawn up in a fine capillary the lower end of which was sealed. The capillary with the blood was kept at 37°C. After one hour the clot was separated ; after two hours the serum was withdrawn, a drop mounted on the slide with a coverslip, both of standard thickness, and the lipoid particles (chylomicrons) in a standard field counted under the dark ground illumination. Typical results are given in the table III.

TABLE III.

Chylomicron count after the ingestion of standard meal containing 1 oz. Fat and 2 oz. Bread.

Hours	Blank	Butter	Ghee	Groundnut oil	Vanaspati	Sesame oil	Coconut oil
0	38·2	19·6	30·5	32·4	5·9	12·7	16·7
1	42·4	19·1	27·1	37·7	16·9	11·6	14·6
2	33·1	191·0	168·0	35·8	77·3	78·2	77·0
3	31·6	104·9	128·5	30·5	76·4	47·0	76·8
4	28·1	140·5	128·0	39·9	115·0	43·1	41·7
5	..	76·2	107·8	206·3	88·9	85·2	23·6
6	..	..	..	122·8	118·7	83·9	..
7	..	..	..	45·8	..	..	..

A number of experiments have been performed on the five subjects mentioned above and the conclusions are as follows:—

(i) Butter, *ghee* and coconut oil were found to be rapidly absorbed, whereas groundnut oil and its partially hydrogenated product and sesame oil were absorbed at a slower rate.

(ii) It is suggested that the rate of absorption of fat from the intestines is determined among other things by the presence of fatty acids of low molecular weight constituting the fat. Quite a considerable alteration in the iodine value of a fat by hydrogenation exerted little influence on its absorption.

2. THE NUTRITIVE VALUE OF MILK AND CURDS PREPARED FROM IT.

An investigation has been undertaken for the purpose of studying the changes which might result in the nutritive value of milk subsequent to its conversion to curds by fermentation. As a preliminary task, the domestic method of preparing curds is being studied for its influence on thiamine, riboflavin and nicotinic acid content. The average figures for milk and curds are given below.

	μg. per gm.		
	Thiamine	Riboflavin	Nicotinic acid
Milk ..	0·31	0·74	1·18
Curds ..	0·37	1·03	0·51

Further work on this problem is in progress.

3. VITAMIN CONTENT OF VEGETABLES.

Ten varieties of the locally consumed leafy vegetables have been subjected to analyses for their carotene, nicotinic acid, ascorbic acid, thiamine and riboflavin content. Five samples of each variety have been collected on different days and from different places. The average results of carotene, nicotinic acid and ascorbic acid content of these vegetables are ready. Further work is in progress.

4. NUTRITIVE VALUE OF PURE BRED STRAIN OF RICE.

An important step in the direction of collaboration between agriculture and nutritional science in India was taken by this Unit when it decided to undertake, in the first instance, an investigation into the vitamin contents of pure bred strains developed by the Department of Agriculture, Government of Bombay. Full cooperation on the part of the Department has been assured. The investigation has just commenced. About 36 samples have been received from various rice breeding stations in the Bombay Province.

5. RESEARCHES ON VITAMIN D.

(a) *Laboratory diagnosis of rickets:*—An experiment has only been concluded in which rickets was induced in young puppies and the course of the disease followed closely by (a) calcium, inorganic phosphorus and protein determinations in serum, (b) estimation of serum phosphatase and (c) X-ray pictures of the distal ends of radius and ulna of the animals at frequent intervals.

This experiment was designed to give information on the feasibility of using the solubility products of $CaHPO_4$ and $Ca_3(PO_4)_2$, either or both, for an early diagnosis of rickets.

(b) *Relation between phosphatase of bone and serum*:—In rickets the alkaline serum phosphatase is found to be considerably increased. Since there is also an increase of phosphatase in the rachitic bone, it has been commonly assumed that the increase in alkaline serum phosphatase is due to a simultaneous increase in bone phosphatase. There are reasons to doubt the validity of this assumption. Hence an investigation has been commenced to provide experimental proof by simultaneous quantitative studies of serum and bone phosphatases in induced rickets supported by histological localization of phosphatase in bone.

In addition to these, investigations on the respiration of normal and rachitic epiphyseal cartilage are being continued.

17. Enquiry into methods of treating sick starving destitutes in Calcutta based on the All-India Institute of Hygiene and Public Health, Calcutta.

The work on this enquiry though mentioned as based at the All-India Institute of Hygiene and Public Health, was carried out at the following places in addition :

1. School of Tropical Medicine, Calcutta.
2. Department of Applied Chemistry, University College of Science, Calcutta.
3. Campbell Medical School and Hospital, Calcutta.

It is further necessary to point out that the nature of the work carried out during the present year is strictly not one of " Enquiry into the methods of treating sick Destitutes " but one on the study of effects of starvation. The change over to the latter type of work was necessitated by the fact that a carefully worked out method of treatment was already evolved and made available to workers in the field, and there was a dwindling number of cases of starvation cases being admitted to the various hospitals. The total available number of autopsy cases for investigation during the year were only 55 compared to 78 in the previous year. The workers in the School of Tropical Medicine in particular had to seek cases for their investigation at Narkeldanga camp and Marwari Relief Hospital. Since the question of evolving a system of treatment was not the major consideration the workers restricted their work to one of scientific study of effects of starvation which formed a part of the activity of the workers even while working out the methods of treament. In this each individual group exercised a great degree of autonomy necessitated by their interests.

The work of the period under review may be given as from the various institutions. As the purpose for which this enquiry was instituted has been served the enquiry has been terminated with effect from November 1945.

I. School of tropical medicine.

1. The Section of Protozoology was mainly interested in the incidence of malaria among the sick destitutes. Blood smears were obtained from

patients admitted at Narkeldanga camp and Marwari Relief Hospital. A total of 1,386 destitutes were examined and 473 showed malarial parasites. 34·1 % or above a third of the cases were infected.

The species distribution was as follows :—

Malignant Tertian	41·5%
Benign Tertian	56·5%
Quartan	2·0%

2. The following represents in a tabular form the type of analysis carried out.

		Cases
Blood.	Basal metabolism	3
	Blood sugar	15
	Non-protein and urea nitrogen ..	16
	Total and individual plasma protein ..	16
	Uric Acid	1
	Cholesterol	3
	Calcium	2
	Van Den Bergh	4
	Glucose tolerance test	3
Urine.	Chemical and microscopical examination	26
	Chloride and urea	3
Stool.	Total split and unsplit fat	3

The results obtained were on the same lines as in the previous year.

II. DEPARTMENT OF APPLIED CHEMISTRY, UNIVERSITY COLLEGE OF SCIENCE.

The workers here studied the Biochemistry of Blood in 30 cases.

The following estimations have been made in oxalated blood, blood plasma, and blood serum under the heads A, B and C respectively.

(A) Total oxalated blood—

(i) Non-protein nitrogen,
(ii) Urea nitrogen,
(iii) Free ammonia nitrogen,
(iv) Uric acid.

(B) Blood plasma—

(i) Percentage of total protein,
(ii) Albumin and globulin.

(C) Blood serum—

(i) Calcium,
(ii) Total protein,
(iii) Viscosity.

From the results connected with the nitrogen picture it appears that non-protein nitrogen and free ammonia nitrogen content of blood increase respectively by 40-320 per cent and 200-3000 per cent over the normal. Whereas the urea nitrogen falls to half the normal value, no significant change is observed in

uric acid level. The high non-protein nitrogen may partly be due to dehydration of blood or accumulation of waste products of metabolism due to renal insufficiency.

All the starvation cases show a low haemoglobin level. The iron content of the blood is also very low.

In most of the starvation cases the blood sugar level varied between 36 and 110 mg. per 100 c.c. blood. It has been shown by others that in starvation cases the glucose tolerance is decreased. Similarly in the case of scorbutic animals glucose tolerance has been found to be decreased.

Regarding the protein value of the blood plasma it is found that the figure for albumin is always low, whereas that of globulin is proportionately high giving total protein almost within the normal range. The protein and viscosity figures of the blood serum are within the normal range with only slight variation in viscosity. As regards calcium there is practically no significant difference between the normal and the starvation cases.

III. Campbell medical school and hospital.

The Pathologist of the School and Hospital made a number of post mortem examinations and passed on the material for histopathological studies to the All-India Institute of Hygiene and Public Health.

IV. All-India institute of hygiene and public health.

The section of Biochemistry, Nutrition and Physiological Hygiene undertook all histopathological work of material obtained mainly from Campbell Medical School and Hospital. During the period under review sections were made of 155 cases.

The report of the study may be conveniently summarised under two main headings.

1. Study of gross abnormalities.
2. Histopathological observations.

1. *Gross abnormalities.*

All the cases under review showed extremely poor nourishment. Skin looked dry, wrinkled in places and of a dirty pale colour. Subcutaneous oedema was one of the frequent findings. Hair—dry. Conjunctiva dry, discoloured and of a dirty white appearance in majority of cases. One or more of the serous cavities such as pleura, pericardium and peritoneum showed collections of clear fluid. The gastro-intestinal tract (i) showed wasting and denudation of the mucous layer in certain places, (ii) was congested and haemorrhagic in certain areas. Liver—(a) Enlarged and congested in majority of cases, (b) nutmeg appearance—14% cases, (c) pale with fatty changes in 10% cases. Gall-bladder distended with bile containing a fair amount of mucus in all the cases; in 2% cases only there were cholesterol stones. Spleen—(a) enlarged and slaty coloured, is cut with resistance in 16% cases, (b) Congested, soft, friable and slightly enlarged in most of the cases. Kidneys—enlargement in 3% cases, majority showed congestion. Heart—the conspicuous macroscopic changes were (a) wasting of the cardiac masculature, (b) in 4% of cases enlargement of the right ventricle and in 10% cases that of left ventricle. Atheromatous changes were found in the abdominal aorta in 12% cases.

Lungs—evidence of oedema and congestion in majority of cases; 10% cases showed lobular consolidation and 15% cases showed tuberculous lesions. Brain—slight congestion of the meninges in 12% cases. Bonemarrow—no gross abnormality was detected.

2. *Histopathological observations.*

The general picture in all the tissues under study was one of congestion and degeneration. The small intestine showed characteristic lesions. The lesions in all the organs may be summarised as follows:—

Liver—(i) Fatty degeneration of the hepatic cells in many cases, (ii) cloudy swelling of the cells in certain cases, (iii) alternate areas of congestion and fatty changes in some, (iv) Haemozoin pigment in malarial cases.

Spleen—(i) Increase in interestitial tissue, (ii) dilatation and haemorrhages in the capillary blood vessels underneath the thickened capsule in some cases.

Intestine—(i) Degeneration of the lining epithelium of the villi in majority of cases, (ii) submucous haemorrhages at places with lymphocytes, (iii) infiltration by monocytes and eosinophiles, (iv) complete absence of mucous layer in some cases.

Pancreas—Degeneration of the islets of Langerhans.

Lungs—Alveoli filled up with oedematous material and mono-neuclear cells, consolidated areas and the tuberculous lungs showed characteristic morbid changes.

Kidney—Intense congestion of the glomeruli in some cases. The renal tubules showed degenerative changes.

Adrenal glands—Cells showed degenerative changes in the cortex.

Cerebral Vessels showed congestion only.

18. Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Laboratories of the Board of Scientific and Industrial Research, University Buildings, Delhi.

During the present year, work has been carried out on the separation and identification of different carotenoid pigments present in different varieties of a number of vegetables and fruits. Seven varieties of leafy vegetables, three varieties of carrots, eleven varieties of mangoes and eleven varieties of oranges have been intensively investigated for various carotenoids occurring in them. Green leafy vegetables showed the presence of five different pigments of which only lutein, β-carotene and pseudo-α-carotene could be so far identified by their absorption spectra. β-carotene has been found to constitute 21·9-42·9% of the total pigments. The values of the different pigments were: Xanthophyll 9·2-25·8 mg. per 100 g., β-carotene 3·05-10·77 mg. per 100 g., Pseudo- -carotene 0·5-1·34 mg. per 100 g., Unidentified pigment No. (1) 0·44-0·66 mg. per 100 g., Unidentified pigment No. (2) 0·35-0·74 mg. per 100 g.

Carrots also showed the presence of five different pigments namely, Xanthophyll, γ-carotene, β-carotene, α-carotene, and neocarotene, the principal pigment being β-carotene. The various pigments in red carrots were: Xanthophyll 0·23 mg. per 100 g., γ-carotene 0·09 mg. per 100 g., β-carotene 8·02 mg.

per 100 g., α-carotene 1·10 mg. per 100 g., neocarotene 0·03 mg. per 100 g. In purple carrots the various pigments were : Xanthophyll 0·30 mg. per 100 g., β-carotene 0·02 mg. per 100 g.

In purple carrots, however, only two pigments could be isolated namely Xanthophyll and β-carotene. The amount of Xanthophyll in the purple carrots has been found to be of the same order as in red and yellow varieties but the amount of β-carotene was very low. Presumably the purple pigment which is water soluble inhibits the synthesis of β-carotene in the plant tissues or putting it in different words, functions of β-carotene are performed by the water soluble purple pigment in this plant tissue.

Basing the estimate of vitamin A potency on the biological activity of constituent members, red carrots give 154 I.U. per g. while purple carrots give only 0·3 I.U. per g.

Different varieties of mangoes also show the presence of five different carotenoid pigments, the principal ones being Xanthophyll and β-carotene which constitute 32·4-68·89% and 26·06-64·0% of the total pigments respectively. The other three pigments are present in relatively small amounts. One of these is pseudo-α-carotene, an isomer of β-carotene and occurs very close to β-carotene in the chromatogram. This has been found to vary from 0 to 0·5%. The other pigment which forms a band just above β-carotene is carotenoid χ according to Kemmerer and Fraps (1943). It varies from 0·78 to 2·76% of the total pigment. One pigment has not been identified. The values of the various pigments are : Xanthophyll 0·79-3·9 mg. per 100 g., Carotenoid χ 0·03-0·11 mg. per 100 g. ; β-carotene 0·48-3·99 mg. per 100 g., Pseudo-α-carotene 0·02-0·26 mg. per 100 g., unidentified pigment 0·01-0·06 mg. per 100 g. The vitamin A potency shows a variation range from 8-69 I.U. per g. In the richer varieties, the figures lie between 32-67 I.U. per g. and for poor varieties 8-16 I.U. per g.

Six different pigments have been separated and estimated in different varieties of oranges and their rind. β-carotene has been found to be very low in all the varieties. The values in oranges were : Pigment No. (1) 0·46-1·18 mg. per 100 g., Pigment No. (2) 0·16-0·72 mg. per 100 g., Pigment No. (3) 0·007-0·20 mg. per 100 g., Pigment No. (4) 0·017-0·02 mg. per 100 g., Pigment No. (5) (β-carotene) 0·02-0·065 mg. per 100 g., Pigment No. (6) (Pseudo-α-carotene) 0·005-0·014 mg. per 100 g. The values in peels were : Pigment No. (1) 2·91-7·69 mg. per 100 g., Pigment No. (2) 0·6-2·27 mg. per 100 g., Pigment No. (3) 0·006-0·06 mg. per 100 g., Pigment No. (4) 0·02-0·07 mg. per 100 g., Pigment No. (5) (β-carotene) 0·04-0·10 mg. per 100 g., Pigment No. (6) (Pseudo-α-carotene 0·009-0·026 mg. per 100 g.

Work is being carried on the identification of the various pigments found in oranges through their absorption spectra.

Some carotenoid pigments have been found to isomerize readily on standing, by the action of light, heat etc. In case of β-carotene, as many as 64 isomers are theoretically possible, of which 12 isomers have been isolated and identified by Zechmeister and Polgar. Some of the isomers are no doubt formed by the action of iodine and HI, but a few result by merely on standing, a reaction which is catalysed by heat and light. The question of isomerisation of β-carotene and other active carotenoids which commonly occur in plants has been investigated. This is of importance also from the point of view of vitamin A activity. The results of this study are not yet complete and will be reported later.

So far as assessment of vitamin A potency is concerned, the two methods (i) basing the estimate on total hydrocarbon pigments present and (ii) separately estimating all pigments present and basing the estimate of vitamin A potency on the biological activity of constituent members give appreciably different results. For example, in a sample of carrots, first method gives 154 I.U. per gram while the second 143 I.U. per gram. The difference may not be of importance from the point of view of practical nutrition or field dietary studies, but it is certainly desirable to know the nature of different pigments which contribute to vitamin A potency in any particular food and the exact contribution of each of them.

19. Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

Experimental hepatic cirrhosis.

1. *Effect of continued feeding of diets deficient in the vitamins of the B_2 complex on the histological and biochemical changes in the liver :*—In this experiment, a group of young healthy rats were fed on a synthetic diet composed of purified casein (18 per cent), purified starch, sucrose, melted butter-fat and salt mixture. Each animal was given in addition supplements of cod liver oil (2 drops of the oil by mouth per day) and 20 μg. of thiamin chloride per day.

The animals were weighed every week. At different intervals, the animals were killed and complete histological and biochemical examination of the liver carried out. Chemical examination included determination of water, fat (unsaponifiable matter + fatty acids) and protein content of the liver and qualitative examination of the liver for the presence of vitamin A.

Some of the animals in this group failed to grow satisfactorily and analysis of the livers showed fatty change. The average values for water, fat and protein content per 100 gms. of fresh liver weight were 66·8, 12·7 and 19·5 gms. respectively. The corresponding figures for the control group were 71·05, 4·27 and 20·76. The rest of the animals were apparently in healthy condition, but on post-mortem examination, the liver still showed the fatty change. Water, fat and protein constituted 71·1, 6·5 and 18·7 per cent of fresh liver weight respectively. The test for vitamin A was positive in all the livers examined. Microscopical examination of the livers is in progress.

2. *Effect of feeding synthetic diet containing 10% protein and devoid of vitamins of the B_2 complex on the liver :*—The diet used in this experiment is similar to that given in the above experiment, except that the level of protein (purified casein) was reduced from 18 per cent to 10 per cent. Marked enlargement of the livers with increase in the fat content was noticed in animals examined during the early part of the experimental period. The weight of the liver in these animals was 11 to 12 per cent of the body weight (normal range 3·8 to 4·1 per cent) and the values for water, fat and protein content per 100 gms. of fresh liver weight were 53, 32 and 14 gms. respectively. The average values for water, fat and protein content of the liver in animals sacrificed at various intervals ranging from 91 days to 200 days on the experimental diet were 70·2, 8·9 and 16·3 gms. per cent respectively.

3. *Effect of low protein intake combined with a deficiency of the vitamin B_2 complex on the liver :*—This series of experiments was started to study the effect

of diets containing inadequate proportion of protein in the basal diets deficient in vitamins of the B_2 complex on the structure and chemical composition of the liver. Two such diets were prepared, one containing 8 per cent and the other containing 5 per cent purified casein in the basal diets. The diets contained a high proportion of sugar and starch and were fed to two different groups of rats.

(a) *8 per cent casein diet*:—There was no mortality in rats receiving 8 per cent casein diet in the first two months of the experiment and the average increase of body-weight during this period was only 10 grammes. The animals were sacrificed at intervals ranging from 72 days to 134 days on the experimental diet. The livers of 10 rats were analysed separately for water, fat and protein content and the average values were water 65·8 per cent, fat 15·0 per cent and protein 14·87 per cent of fresh liver weight. Positive colour reaction of vitamin A with antimony trichloride was obtained in the ether extract of all the livers examined.

(b) *5 per cent casein diet*:—Rats receiving 5 per cent casein diet with supplements of cod liver oil and thiamin chloride maintained their original body-weight for about a month and, afterwards, gradually declined in weight. Symmetrical dermatosis of the paws, suggestive of vitamin B_6 deficiency, was noticed in many animals in this group. Owing to the poor general condition, all the animals in this group were killed at intervals ranging from 37 days to 94 days from the commencement of the experiment. On post-mortem examination, the livers were pale yellowish in colour and chemical analysis showed increase in the total fat content. The average values for water, fat and protein content per 100 gms. of fresh liver weight were 64, 17·6 and 14·93 gms. respectively.

4. *Effect of varying levels of protein intake in an otherwise adequate diet on the histological and biochemical changes in the liver*:—In this series of experiments, varying levels of protein were included in the basal diet which was supplemented with all the essential vitamins in the form of cod liver oil (2 drops of the oil by mouth per rat per day) and 0·5 gms. of whole dried brewer's yeast. Four different groups of rats were fed on diets containing 18 per cent, 10 per cent, 8 per cent and 5·0 per cent respectively of purified casein.

(a) *Effect of feeding 18 per cent casein diet supplemented with yeast*:—The animals in this group showed good growth and there was no evidence of any gross deficiency. Analysis of the livers showed that irrespective of the duration of the experimental period, the proportion of moisture, fat and protein was more or less constant. The average values were 71·05, 4·27 and 20·26 gms. per cent respectively.

(b) *Effect of feeding diet containing 10 per cent casein supplemented with yeast*:—As in the previous group, the animals in this group were healthy and showed satisfactory growth. The weight of the liver was proportional to body-weight and the organ was normal in appearance. The percentage of moisture, fat, protein and glycogen (estimated by the method of Good *et al*, 1933) were 72·01, 5·15, 19·03 and 1·26 respectively.

(c) The experiments on the effect of feeding diets containing 8 per cent and 5 per cent casein respectively supplemented with yeast are in progress.

PREPARATION OF METHIONINE.

A small quantity of pure methionine has been isolated from casein hydrolysate following the method described by Hill and Robson (1934). But the yield being very low, we are trying to synthesise it in our laboratory.

20. Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

FATE OF ADDED IRON TO EGG-YOLK.

It has been previously shown that whole egg-yolk (egg-yolk mentioned here and subsequently in the report is of duck if not otherwise mentioned) can take up added iron in ferric condition up to the extent of 10-12 mg. but it fails to take up any iron when iron is added in the ferrous state. In order to see whether this added iron goes to form some stable complex with the protein of egg-yolk or it is loosely bound, the following observations have been made.

The absorption of iron by egg-yolk cannot be disturbed by some enzyme inhibitors such as iodoacetate, malonate, flouride and by narcotics such as urethane, chloretone, chloral. But with cyanide, there is a definite inhibition to the extent of 10%.

The absorbed iron cannot be eluted at different pH's or by dilute cyanide solution and is not dialysable.

Moreover, the absorbed iron cannot be detected in the hydrolysate on enzymatic digestion for a prolonged period of seven days. The assay was made on the hydrolysate at intervals of 24 hours.

From these observations it is apparent that added iron goes to form some stable complex with protein of egg-yolk and is not loosely bound. A similar phenomenon of absorption of iron has been observed by Tompsett (Biochem. J. 1934, **28**, 1804) while working with biological materials and he has concluded that iron forms stable complexes with phospholipins and phosphoproteins.

NATURE OF IRON IN EGG-YOLK.

It has been observed by Mcfarlane (Biochem. J. 1932, **26**, 1063) and also by ourselves that iron is in stable combination with proteins of egg-yolk and cannot be liberated by treatment with protein precipitants such as trichloroacetic acid. In contrast to this view, Hill (Proc. Roy, Soc. Lond. B107, 1931, 205) however, held that iron is in inorganic state, either as ferric iron or as colloidal ferric hydroxide. This hypotheses can, however, be tested. If iron remained as free ferric iron it can then be dialysed out under suitable condition. Experiments, however, showed that iron of egg-yolk could not be dialysed out on prolonged dialysis for a week through parchment paper or vitellin membrane of egg-yolk. Again if iron were in colloidal hydroxide state, it would be extractable with hydrochloric acid which was not, however, borne out by our experiments, as no iron was found in the acid filtrate. Again iron of egg-yolk could not be liberated on enzymatic hydrolysis.

All these experimental evidences seem to throw some doubt on the validity of the claim put forward by Hill and support the view that iron of egg-yolk is in a stable combination as protein complex.

Iron containing complex in egg-yolk.

Mcfarlane (Biochem. J. 1932, **26**, 1063) has shown that the lecitho-vitellin fraction of hen's egg-yolk contains iron to the extent of 45 mg. %. Saha and Mazumder (unpublished) have shown that total iron of egg-yolk can be assayed in lecitho-vitellin fraction of proteins provided it is precipitated at its optimum coagulation temperature, 70°C. It has, however, been found by ourselves that lecitho-vitellin can be completely precipitated on keeping overnight at 0°C. and the total iron of egg-yolk can be estimated in such a preparation.

Iron of the lecitho-vitellin cannot be detected in protein free-filtrates after treatment of a solution of lecitho-vitellin in sodium chloride with trichloroacetic acid, nor can iron be extracted with hydrochloric acid, nor it is dialysable. Moreover, the iron of the protein is not liberated in the hydrolysate on enzymic digestion. These observations lead to the conclusion that vitellin iron behaves in the same way as iron of the egg-yolk and afford further proof of the fact that the total iron of egg-yolk is in organic combination with lecitho-vitellin.

Behaviour of other metals in egg-yolk (copper).

As the influence of copper on the utilisation of iron to form haemoglobin is well-known it was considered worth while to study egg-yolk with respect to copper.

It has been observed by ourselves that egg-yolk contains 0·0083 mg. of copper. The copper of the egg-yolk can be liberated in the filtrate by treatment with protein precipitants such as trichloroacetic acid. Furthermore, when copper is added to egg-yolk in the cupric state, egg-yolk fails to take it up and the added copper can be totally estimated in the protein free filtrate and can be completely dialysed out in 48 hours. All this behaviour of copper is quite in contrast to that of iron. However, just like iron of egg-yolk, copper cannot be dialysed out in 48 hours.

It should be pointed out that similar behaviour of iron and copper has been observed in other biological systems and preparations. Tompsett (Biochem. J. 1934, **28**, 1536, 1544) working with blood plasma has shown that whereas plasma iron cannot be detected in protein-free filtrate copper of plasma can be estimated in the filtrate. Iron and copper of the Iron-Copper-Nucleoprotein complex (Ann. Biochem. Expt. Med. 1941, **1**, 195) also behave similarly.

Iron and copper in egg white.

To complete the study of the egg as a whole, similar observations have been made with egg-white. The egg-white contains definite but a small amount of iron. For duck's egg-white the value for iron is 0·0052 mg. while the corresponding value for hen's egg-white is 0·0029 mg. The iron is in ferric condition and can be estimated by α-α' dipyrydyl only upon reduction with hydrosulphite and acetic acid. The iron in egg-white, unlike that of iron in yolk, is extractable with trichloroacetic acid and dialysable. All these indicate that iron in white is but loosely held.

The egg-white contains traces of copper and on addition of diethyldithiocarbamate a faint yellow colour develops. But the colour is too faint to allow matching in a colorimeter.

21. Enquiry on poor rice diets and food preparations in common use in South India under Miss D. Pearson at Women's Christian College, Madras.

Experiments with rats have been continued on the same model as previously reported by Dr. Mason (1). This year experiments have been conducted for nine weeks and the growth has been statistically analysed. Preliminary experiments on the relation of butter as a supplement to a poor rice diet have been sent for publication to the Indian Journal of Medical Research (2), (3). Further experiments have been carried out in this subject but the results are not sufficiently conclusive to be published at this stage.

From these experiments there are certain facts that we can report on.

TABLE I.

Effects of Supplements to the Poor Rice Diet.

Supplement	No. of rats	Amount of supplement gms/rat/day	Average weekly gain in weight in gms. (8 weeks)
Nil	59	..	2·07
Milk	13	12·500	9·80
Curds	2	12·500	8·15
Calcium lactate	18	0·115	5·77
Casein technical	8	0·400	3·22
Casein vitamin free	2	0·400	2·50
Technical casein+calcium lactate ..	6	·4+·115	8·93
Butter	27	0·500	1·58
Butter	5	1·000	0·81
Groundnut oil	2	0·500	2·25
Roasted groundnuts	6	1·000	3·80
Defatted groundnuts	2	0·600	5·15
Dhal arhar (Red gram husked) ..	5	2·000	4·15
Dhal arhar „ „ „	4	3·000	5·39
Sprouted green gram	2	3·000	2·80
Ragi	2	4·600	6·63
Yeast—brewer's sun dried	2	0·800	7·90
Egg yolk	2	1·500	7·75
Egg yolk	2	1·000	5·40
Egg white	2	1·000	3·10
Whole egg .. .. " ..	2	1·000	4·38
Amaranth	8	5·000	5·43

(1) Report of the Scientific Advisory Board, 1944.

(2) Mason, Theophilus, Frimodt-Moller—The influence of butterfat in the absence or presence of Casein on young rats on a rice diet—I.J.M.R., October 1945.

(3) Mason, Devadas, Frimodt-Moller—The Effect on Growth in Rats of butter and ragi (Eleusine Coracana) separately and combined as supplements to the Poor Rice Diet of South India (sent for publication to I.J.M.R.).

From this it will be seen that due to the improved supply of rice received this year, the average weekly gain in weight of the rats on the 'Poor Rice Diet' has increased from 1·70 to 2·07.

A comparison of the Diets used as a basis for our experiments has been made, and the results are as follows.

TABLE II.

Comparison of Diets.

Diet	No. of Rats	Average weekly increase in weight in gms. (8 weeks)
Poor Rice Diet	59	2·07
Rice 2/3 + Ragi 1/3	10	7·72
Rice 5/6 + Ragi 1/6	2	6·70
Experimental School Diet*	19	10·00
Wheat Diet	2	4·63
Good Stock Diet	16	16·00

*See Report for 1944 for composition.

NOTE.— The wheat diet is the same as the 'Poor Rice Diet' except that wheat is substituted for the rice.

Last year a modification of the 'Poor Rice Diet' called the Experimental School Diet was reported. An experiment was carried out to see the effect of substituting the dhal wholly or partially with groundnuts and groundnut cake (Mysore). Dhal is 10% of the diet.

TABLE III.

Modification in Experimental School Diet.

Modification	No. of Rats	Average weekly increase in weight in grams (8 weeks)
E.S.D. = 10% Dhal		
Control	2	12·97
No Dhal — Raw Groundnuts 10% ..	2	9·35
No Dhal — Raw Groundnut Cake 10% ..	2	8·45
Dhal 6% + Groundnuts 4%	2	9·28
Dhal 6% + Groundnut Cake 4%	2	10·72

Groundnut cake is not significantly better than whole groundnuts for the promotion of growth, while dhal is superior to either. Therefore, although when protein is scarce groundnuts and groundnut cake are valuable additions to the diet, it is much better if the diet also contains some dhal. Moreover in substituting groundnuts in cooking recipes we have not succeeded in getting good results if we substitute more than half the dhal with groundnut cake.

TABLE IV.

Effects of supplements to Experimental School Diet.

Supplement	No. of rats	Amount of supplement gms/rat/day	Average weekly increase in weight in gms. (8 weeks)
Nil	19	..	10·00
Milk	8	12·500	14·00
Roasted Groundnuts ..	4	1·000	9·60
Butter ..	4	0·500	7·77
Butter	2	1·000	5·60

South Indian Food Preparations.

The same procedure as in the report for 1944.

TABLE V.

Analyses of South Indian Food Preparations.

Food Preparation	Moisture gm. %	Protein gm. %	Fat gm. %	Mineral matter gm. %	Calcium gm. %	Phosphorus gm. %	Iron mgs/100 gms.	Calories per 100 gms.
Vadai—2 more hotel samples. (Black gram, green chillies, onions, curry leaves, coriander leaves, salt, fried in Dalda.)	42·08	12·26	15·45	1·661	·0483	·1213	11·72	300
Iddaly—complete analyses. (Parboiled milled rice, black gram and salt.)								
Mean of Hotel samples	69·75	4·177	·0153	·7764	·0193	·0453	3·483	100
"Home made" samples	68·04	4·249	·0276	1·240	·0181	·0270	6·49	126
Omapodi–(Bengal gram, dhal, raw rice, omum, salt and any oil).								
Summary of Hotel samples	2·136	14·45	35·68	4·024	·079	·1130	18·05	559
"Home made" samples	3·443	14·88	23·14	3·114	·0505	·131	17·14	494
Dhosais–(Parboiled rice, black gram, salt and ghee or oil).		"						
Mean of Hotel samples	46·88	6·785	10·478	1·387	·0402	·059	7·828	266
Mean of Home made samples	62·90	5·636	.0335	1·598	·0223	·038	6·77	107
Bajjis—vegetable plantain, Bengal gram, dhal flour, rice flour, dry chillies, turmeric, salt, soda bicarb and fried in some oil or ghee.								
2 Hotel samples	40·36	7·64	22·46	4·120	·0378	·0717	16·6	181·0

TABLE V. (*Contd.*)

Food preparation	Moisture gm. %	Protein gm. %	Fat gm. %	Mineral matter gm. %	Calcium gm %	Phosphorus gm. %	Iron mgs./100 gms.	Calories per 100 gms.
Tomato—vegetable tomato, others same. 2 Hotel samples	42·20	5·512	22·27	2·047	·0632	·0691	10·6	254
Onion—vegetable onion, others same. 2 Hotel samples	44·40	6·941	21·45	3·099	·0460	·1017	9·8	222
Brinjal—1 Hotel sample	47·91	6·612	17·32	2·019	·0345	·0895	10·2	174
Brinjal. Home made.	30·73	6·216	41·78	2·063	·0400	·1010	6·7	236

Other analyses carried out in this laboratory.

TABLE VI.

Analyses of other foods.

Sample	Moisture gm. %	Protein gm. %	Fat gm. %	Mineral matter gm. %	Calcium gm. %	Phosphorus gm. %	Iron mgs./100 gms.	Calories per 100 gms.
Parry's Food Yeast	7·173	40·8	1·34	4·80	·072	·135	76·9	276
Groundnut Cake (Mysore Government)	12·24	52·5	4·92	3·19	·078	·043	12·0	274
Sambar	84·87	2·78	1·303	1·823	·0228	·0217	3·6	61

22. Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary protein to blood and muscle haemoglobin under Professor Damodaran at the University Biochemical Laboratory, Triplicane, Madras.

Experiments have been carried out to determine the relative efficiencies in blood regeneration of the following proteins fed at a uniform level of 5% : casein, egg albumin, wheat gluten and rice protein. The rate of blood regeneration with casein and egg albumin when fed at different levels has also been studied.

The experimental procedure was to make the animals anaemic by injection of phenylhydrazine and then to study the rate of recovery from anaemia by periodic determination of R.B.C. count and haemoglobin content of blood on a diet containing the particular protein under examination. As has been previously shown in this Laboratory a consistent and readily reproducible type of anaemia can be induced in albino rats by a single intraperitonial injection of phenylhydrazine administered at the rate of 2 mg. per 100 g. of body weight. This treatment causes an acute anaemia which reaches its peak between three

and four days after injection, the R.B.C. being reduced to about 50% and the haemoglobin to about 60% of the initial values. The injection causes no other abnormalities and when the animals are kept on a complete diet the blood picture is rapidly restored to normal. It was the object of the present experiments to compare the rates of recovery from the anaemia of animals receiving a protein-free basal diet when supplemented with different proteins. The basal diet was of the following composition :

Starch	65%
Sugar	16%
Butter fat	10%
Salt mixture (Steenbock's)	4%

Arrow-root starch with a protein content of 0·3% was employed. Vitamin supplements were given in the form of shark liver oil and yeast. B.D.H. light white casein and B.D.H. egg albumin were used. Wheat gluten was prepared in the Laboratory from whole wheat flour by the usual method. In the case of experiments with rice protein a diet containing 5% of the protein was obtained by substituting for starch 70% of rice flour from polished raw rice with a protein content (N × 6·25) of 7·4%. The animals (white rats weighing between 130 and 150 gms. and divided into groups of six each) were put on the diets 3 days prior to the injection of phenylhydrazine. Blood was obtained by snipping the tail. R.B.C. and haemoglobin values were obtained daily for the first three days on the experimental diet, the average for the three days being taken as initial values. After injection of phenylhydrazine R.B.C. count and haemoglobin determinations were made every fourth day.

TABLE I.

Increase of R.B.C. and Haemoglobin in a period of 8 days at 5% levels.

	Casein				Egg albumin				Wheat gluten				Rice protein			
	4th day	12th day	Inc.	% Inc.	4th day	12th day	Inc.	% Inc.	4th day	12th day	Inc.	% Inc.	4th day	12th day	Inc.	% Inc.
R.B.C. in millions per c.mm.	3·40	5·51	2·11	62·1	3·35	5·77	2·42	72·4	3·48	4·75	1·27	36·9	3·66	5·05	1·39	38·0
Haemoglobin in gms. per 100 cc.	8·80	13·72	4·92	55·9	9·04	14·29	5·25	58·1	9·14	12·41	3·27	35·8	8·84	12·27	3·43	38·8

In Table I are given the results of experiments in which casein, egg albumin, wheat gluten and rice protein were fed at the same level namely 5% by weight. The figures given are for the first part of the experimental period, for eight days from the onset of severe anaemia (fourth day after injection of phenylhydrazine). It is clear that there are marked differences in the haemopoietic activity of these four proteins, egg albumin and casein falling into one class with high haemopoietic activity, while wheat gluten and rice protein resemble each other and

are much less efficient. Thus on a casein diet, in the first 8 day period from the stage of acute anaemia the increases in haemoglobin and R.B.C. are 55·9% and 62·1% respectively. Corresponding values for wheat gluten are 35·8% and 36·9% respectively. Differences of the same kind exist between egg albumin and rice protein. The animals are restored to their normal blood picture in 12 days on casein and in 11 days on egg albumin. The corresponding periods on wheat gluten and rice protein are 21 and 20 days respectively.

Level of protein intake and haemoglobin regeneration.

Casein and egg albumin were fed to different groups of animals at the following levels:

Casein	2%	3%	5%	15% &	20%
Egg albumin	2%	3%	5%	15% &	20%

From the records of food intake it was found that there was little variation in the amounts of food consumed with different diets so that the protein content of the food mixture could be taken to be a fair index of the amount of protein consumed.

TABLE II.

Increase in R.B.C. and haemoglobin in 8 days at different levels of casein and egg albumin.

Expt. No.	Protein level	No. of animals	R.B.C. in millions per c.mm.				Haemoglobin in gms. per 100 cc.				No. of days taken to come to normal
			4th day	12th day	Inc.	% Inc.	4th day	12th day	Inc.	% Inc.	
1.	Protein-free	6	3·31	4·09	0·78	23·6	8·19	10·81	2·62	32·0	25
	Casein										
2.	2%	6	3·36	4·43	1·07	31·8	8·60	12·35	3·75	44·2	18
3.	3%	6	3·59	5·57	1·98	61·7	8·77	13·36	4·59	55·1	12
4.	5%	6	3·40	5·51	2·11	62·1	8·80	13·72	5·12	59·5	12
5.	15%	6	3·62	6·66	3·04	86·1	8·31	14·48	6·17	74·7	10
6.	20%	6	3·61	7·20	3·59	100·0	8·60	15·11	6·51	75·6	8
	Egg albumin										
7.	2%	6	3·50	4·61	1·11	31·4	8·04	11·35	3·31	42·5	18
8.	3%	6	3·60	5·79	2·19	63·5	8·68	12·60	3·92	48·4	13
9.	5%	6	3·35	5·77	2·42	67·0	9·04	14·29	5·62	58·1	11
10.	15%	5	3·51	6·92	3·41	95·7	7·82	14·37	6·55	84·6	10
11.	20%	6	3·75	7·43	3·68	99·6	8·61	15·4	6·79	88 5	8

From the results of the experiments summarised in Table II it is obvious that blood regeneration is considerably influenced by the level of dietary protein.

While the animals on 2% casein or egg albumin take 18 days to show a normal blood picture on 20% of either protein recovery from anaemia is complete in 8 days. There is no reason to assume that even at the 20% level the maximum possible rate of regeneration was attained. Experiments to ascertain if more accelerated haemoglobin formation can be secured by still further raising the protein level are now in progress.

LEPROSY

23. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

The work during the year has included clinical, therapeutic, histological, bacteriological and immunological studies.

Clinical.

(1) *The long term study.*

The long term study of selected cases of leprosy has been continued. Several of these cases have now been under study for three years or more, and others for a shorter period. It is yet too early to arrive at any definite conclusions, the following general observations may however be made.

Cases of doubtful classification. A large majority of cases of leprosy can be classified as neural or lepromatous on clinical grounds. There are, however, a small percentage of cases in which the clinical findings are not clear cut, lesions being typical neither of the tuberculoid nor of the lepromatous type, but having some features of both the types. It is difficult or impossible to make a proper classification or prognosis in these cases.

The lesions in these cases resemble the 'tuberculoid' lesions in being localised, but unlike the tuberculoid lesions they are smooth and shiny, and are strongly positive bacteriologically.

The result of the lepromin test appears to be of great value in sorting out these cases. In the lepromin positive cases in this group the lesions were histologically tuberculoid, though not quite typical; in most cases they have since subsided, and in no case have they become lepromatous. In the lepromin negative cases the lesions were histologically either lepromatous or doubtful; the disease has progressed in many cases, the lesions having become frankly lepromatous in several cases.

Flat patches of the neural type with ill-defined margins. The simple flat patches of the neural type usually have well-defined margins. In some cases, however, margins are ill-defined, and consequently the patches are not so well circumscribed. Such cases are believed to be more likely to pass on to the severe lepromatous type.

In none of the cases under study has the disease so far changed into the lepromatous type. In a small number of cases the disease has progressed, and fresh lesions have appeared; in some it has remained stationary; and in others the lesions have faded slightly or to a great extent. All the cases in which improvement has been noticed, belong to the lepromin positive group, and the majority of the cases in which the disease has progressed belong to the lepromin negative or doubtful group.

Tuberculoid cases in a stage of reaction. In this group are included 'tuberculoid' cases in which the history of the disease definitely indicated the presence of reaction, that is, the disease had existed for some time but the signs of exacerbation were present only for the previous few months.

Clinically the lesions in some of the cases were typically tuberculoid with well defined margins, while in others the lesions were smooth with shiny surface and an ill-defined margin. Histologically all the lesions were tuberculoid,

though not typical. Bacteriologically some of the cases were positive. They were all lepromin positive.

In all the cases the lesions have shown considerable subsidence and in some they have become completely flat. With the subsidence, the lepromin test has become weaker, and the tuberculoid histology in some cases has been replaced by the non-specific round cell infiltration.

Cases of leprosy amongst children. It is often said that in children the prognosis of leprosy has to be a guarded one. However, it appears that in case of 'tuberculoid' lesions the prognosis amongst children is as good as amongst adults. Of the tuberculoid cases amongst children under study, a small number were lepromin negative, and a large number lepromin positive. In the smaller lerpomin negative group, the condition has remained the same, or the disease has progressed. In the larger lepromin positive group, thick lesions have become flat in many cases, and in several instance the patches have faded almost completely.

(2) *Erythrocyte sedimentation rate.*

A study is being made of the value of erythrocyte sedimentation rate as a help in regulating the dose of the hydnocarpus preparations in the treatment of leprosy. In a number of cases, representing both the types of the disease, sedimentation rate is ascertained every week, but the dose of the hydnocarpus oil is prescribed without any reference to the result of the test.

In the neural cases, the sedimentation rate is usually low, being below 20. This low rate is more or less maintained, but at times, because of the occurrence of some intercurrent disease, it becomes temporarily higher. In such cases the dose of the oil is decreased because of the intercurrent clinical condition, but without any reference to the result of the test. In certain cases, a rise in the index is sometimes seen without any change in the clinical condition, and in such cases, the dose is not lowered, but maintained or increased, without any untoward effects.

In the lepromatous cases, the sedimentation rate is usually, though not always, rapid, the index being high. In some of these cases, with the onset of reaction or some intercurrent disease, the index becomes higher. In these cases, the dose of the oil is reduced, in view of the clinical condition of the patient, without any reference to the results of the test.

It appears that the results of the sedimentation test do not give any information, not given by the clinical condition of the patient. The test does not, therefore, appear to be of any value in regulating the dose of the hydnocarpus preparations.

THERAPEUTIC.

(1) *Penicillin.*

Penicillin has been tried in white rats infected with rat leprosy bacilli. 24 rats were inoculated with an emulsion rich in rat leprosy bacilli, and were immediately put on penicillin treatment, 3 injections a day of 100 units each. In case of rats that did not die earlier, the treatment was continued for seven months. Three rats died within three months, a period not sufficient for the development of the generalised rat leprosy infection. The remaining 21 rats died from three to seven months after inoculation, and all of them, showed

gross generalised lesions. Thus the treatment with penicillin did not prevent the development of generalised infection.

Penicillin has been tried to a limited extent in human cases of leprosy without any beneficial effects. This is in keeping with the results recently reported from America, where penicillin was given to several cases of leprosy in doses of 50,000 to 100,000 units daily, for a month, without any beneficial effect.

(2) *Stilbamidine.*

A study has been made of stilbamidine in experimental rat leprosy. Stilbamidine has been found to be of great value in the treatment of kala-azar, and there is evidence that there is some thing common between the causative organism of kala-azar and the acid-fast bacilli. A study has, therefore, been made of the value of stilbamidine in rat leprosy as a preliminary to its being tried in human cases.

10 rats inoculated with emulsion rich in rat leprosy were given 1 mg. stilbamidine twice a week, the longest period of administration being 6½ months. 2 died within three months of inoculation, six died from three to seven months after inoculation, and one is still living. In none of the animals has any macroscopic evidence of infection been found in liver, spleen omentum, etc., and microscopic examination of these organs have shown the presence of only a small number of bacilli.

(3) *Sulphonamide derivatives.*

Dr. Rajagopalan has prepared pure chaulmoogric and hydnocarpic acids for ultimate use in the synthesis of chaulmoogric and hydnocarpic derivatives of the sulphonamides. The feeble activity of the familiar sulphonamides towards mycobacterial diseases, notwithstanding their efficacy in some other bacterial infections may be related to the high lipoid content of these bacilli; possibly, the outer waxy layer of these organisms prevents the access of the drugs into their interior. It is possible that lipophilic derivatives of the sulphonamide drugs may be active against acid-fast bacilli. In this connection the chaulmoogric and hydnocarpic derivatives of the sulphonamides are of special interest to leprosy workers; by incorporating the active principles of chaulmoogra oil in the sulphonamide molecule, it may be possible to get the combined effect of the two well-known remedies, one of which has been the mainstay in the treatment of leprosy for centuries, and the other has been proved to be very effective in other bacterial diseases.

Preliminary experiments have been undertaken for the preparation of di-amino-diphenyl-sulphone, the parent drug of 'promin' and 'diasone', which have been reported on more favourably in the treatment of leprosy than the familiar sulphonamides.

Bacteriology.

1. *Attempts at cultivation.*

Attempts have been made to grow leprosy bacilli on certain media recently reported to have been used successfully. The reported results have not been confirmed.

(2) *Value of nasal smears.*

An investigation has been started to find out if leprosy bacilli are ever

found in the nose of cases of the neural type of leprosy in whom the skin lesions are bacteriologically negative. The definition of the neural type of leprosy, adopted at the International Leprosy Congress, Cairo (1938) includes the following statement " Bacteriologically the skin lesions are typically but not invariably found negative by standard methods of examination, though the nasal mucosa may be positive ".

The matter is being studied in both the ' new ' cases who attend for the first time for diagnosis and treatment, and in ' old ' cases who have been taking treatment for some time. Of the 600 ' new ' neural cases, with bacteriologically negative skin lesions, bacilli were not found in the nose in even a single case; of the 150 ' old ' cases, at present with bacteriologically negative skin lesions, leprosy bacilli were found in the nose in only two cases, and those two cases had previously been bacteriologically positive in the skin.

Histology.

(1) *Lesions of the ' Tuberculoid ' type.*

A study has been made of the lesions of the tuberculoid type in the various phases of activity. The study has shown that typical tuberculoid histology may not be seen during all the stages of activity. On the one hand, during phases of increased activity (reaction), while the tuberculoid nature of the granuloma is apparent, the histology may not be typical; on the other hand, with subsidence of activity, there is a tendency for the tuberculoid element to disappear, and with complete subsidence, there may be found only non-specific granuloma with round cell infiltration.

(2) *Lesions of the lepromatous type.*

A study has been made of the histology of the different kinds of lesions of the lepromatous type. It appears that the presence of multi-nucleated giant cells in typical lepromatous lesions is by no means a rare finding.

(3) *Other studies.*

Histological studies have been made in connection with the clinical studies outlined above.

Immunology.

(1) *Protein fractions of acid-fast bacilli.*

The study of the protein fractions of the acid-fast bacilli has been continued. This study has been made in the hope that an antigen sutiable for work in leprosy may be isolated from cultures of an acid-fast bacillus. The results of the study were promising, but so far it has not been possible to obtain a fraction which produces results uniformly identical with those produced by ' lepromin ' prepared from the leprous lesions of patients. The nearest approach to the ' lepromin ' has so far been the acid-soluble fraction of the Stefansky's bacillus, the causative organism of rat leprosy, but even this fraction does not give uniformly negative results in the lepromatous cases.

(2) *Filtrate from lepromin.*

It was previously believed that filtrate from lepromin suspension was antigenically inactive. Some time previously it was reported under this

enquiry that filtration reduces activity, but does not destroy it. Fernandez reported that if the filtrate be concentrated by reducing its volume on a water bath, it is as active as the ordinary lepromin. This matter has been investigated, and the report of Fernandez has been confirmed.

24. Leprosy enquiry under Dr. R. G. Cochrane at the Lady Willingdon Leprosy Sanatorium, Chingleput, Madras.

The work of the enquiry was continued under the following heads :—

(1) Epidemiological and preventive
(2) Clinical and pathological
(3) Therapeutic
(4) Animal.

Owing to the transfer of the officer-in-charge of the leprosy enquiry from Chingleput to Vellore there has been a certain amount of inevitable dislocation of the work and therefore the enquiry has lacked the continuity which it otherwise would have had. It is hoped that this defect will be rectified under the new proposals which have been submitted for the continuation of the enquiry as part of the research activities of the Honorary Director of the Leprosy Campaign, Madras Presidency.

Epidemiological and Preventive :—Field work has continued in the North Arcot District of the Madras Presidency. Dr. Sarma, Field Investigation Officer in the British Empire Leprosy Relief Association up to the time of his transfer to the staff of the Honorary Director, Leprosy Campaign, carried out survey work in the border between South and North Arcot. The observation of the previous year that the greatest factor in the spread of leprosy and the establishment of a new focus is contact, usually as a result of marriage was confirmed. The following table gives the incidence in the villages surveyed.

DESCRIPTION OF THE VILLAGES AND THEIR INCIDENCES.

TABLE I.

Village		Gross incidence	Distance from heavily infected place.
I. *Vaipur area.*			
1. Vaipur	Caste	65·0 per 1000	
2. Vaipur	Cheri	No leprosy	100 yards
3. Esikili	Caste	137·5	5 miles from Vaipur
II. *Muttathur area.*			
1. Muttathur	Caste	15·5 per 1000	
2. Do.	Cheri	10·7	1 furlong
3. Nemur	Cheri	39·0	
4. Nemur	Caste	5·9	2 furlongs
5. Mandagapattu	Caste	30·2	
6. Do	Cheri	Nil	2 furlongs

TABLE I. (*Contd.*)

Village		Gross incidence	Distance from heavily infected place.
III. *Melkarni area*			
1. Melkarni	Caste	38·36	
2. Do	Cheri	No leprosy	1 furlong
3. Vembi	Caste	34·0	3 miles
4. Kundalapuliyur	Caste	48·4	3 miles
5. Do	Cheri	27·8	2 furlongs
6. Kottiyampundi	Caste	47·56	5 miles from Melkarni

It is interesting to note whereas the caste villages of Vaipur, Melkarni and Mandagapattu have a heavy incidence of leprosy, the cheris have no leprosy at all. In reviewing these figures along with statistics from last year it was noticed that villages which maintained close marriage relationship with other villages already infected usually show a high incidence of leprosy. For instance, in this group of villages Esikili, with a very high incidence of 137·5 per 1000, consists of one caste whose marriage relationship was mainly restricted to Vaipur and as a result open cases from Vaipur who were related by marriage to persons in Esikili were constantly found in this village, and there appears to be no repugnance in arranging marriages between open cases and young girls. The more detailed statistics of this village are worth noting.

TABLE II.

No.	Village	Caste	Gross incidence	Child rate	Open case rate	Remarks
1.	Esikili	Caste	137·5	27·2%	22·2%	One caste of Odayars
2.	Do	Cheri	Incomplete			
3.	Vaipur	Caste	65·0	38·3%	30·0%	Do
4.	Do	A.D.	No leprosy			
5.	Kundalapuliyur	Caste	48·43	31·85%	40·9%	
6.	Do.	A.D.	27·8″	22·2%	50·0%	
7.	Melkarni	Caste	38·36	31·7%	45·0%	
8.	Do	A.D.	No leprosy			
9.	Kottiyampundi	Caste	47·56	32·6%	51·2%	One caste of Goundans.
10.	Anantapuram	Caste	30·2	26·1%	16·7%	
11.	Vembi	Caste	34·0	18·2%	50·0%	
12.	Mandagapattu	Caste	30·2	37·0%	51·9%	Goundans
13.	Do	Cheri	No leprosy			
14.	Nemur	Cheri	39·0	36·3%	36·3%	Small cheri of 282 population
15.	Do	Caste	5·9	Too low for calculation		
16.	Muttathur	Caste	15·5	11·1%	44·4%	
17.	Do	Cheri	10·7	Too low for calculation.		

It is felt that the most usual way for leprosy to become heavily established in a village is not by spread through casual contact, water or any of the more indirect methods, but through marriage. Thus the most intimate family relationship is set up, and the condition for acquirement and spread of leprosy are most favourable. In many instances the villagers become so interrelated that they can be considered a single family unit infecting each other's children. The findings both this year and last lead us to the tentative conclusion that while the gross incidence, child rate and open case rate must be taken into consideration in estimating the seriousness of leprosy in a village, two other factors may have to be taken into account i.e., the percentage of the total number of cases in the 0-34 age groups and the percentage of lepromatous cases in these age groups. In certain villages the sex ratio was markedly reversed from the usual 3 to 4 males to one female to 1·4 males to one female, the significance of this in relation to the epidemiology of leprosy is not yet clear.

Epidemiological work has continued at the Silver Jubilee Children's Clinic at Saidapet and the tendency to the spontaneous disappearance of neural lesions in childhood has been confirmed. The following table gives a summary of the following work of over the past six years.

TABLE III.

Year	Total	(a) Much improved		(b) Lesions disappeared		(c) Residual	
		No.	Percentage	No.	Percentage	No.	Percentage
1937	67	44	65·7	30	68·18	14	31·82
1938	39	25	64·2	12	48·0	13	52·0
1939	31	19	61·3	10	57·89	9	42·11
1940	31	17	54·8	7	41·18	10	58·82
1941-44	81	19	23·46	4	21·05	15	78·95
Grand total	249	124	49·8	63	50·8	61	49·2

NOTE :—Column (a) indicates the total percentage which have shown great improvement. Columns (b) & (c) divide the number in column (a) into children in which the lesions have disappeared and residual lesions, that is lesions which have remained inactive but are still visible.

It appears that in S. India leprosy is frequently a benign non-progressive disease in childhood and that over 50% of all children who acquire the disease only a relatively small number progress to the more serious forms.

A further survey was completed in another area of Saidapet and the following tables give a summary of the findings.

TABLE IV.

Name of the village Area 'C' Taluk Saidapet Chingleput District.

		Total	Male	Female
Total population	Adult	1850	938	912
Number examined	Adult	1730	867	863

TABLE IV (*Contd.*)

Name of the village Area 'C'	Taluk Saidapet	Chingleput District.		
		Total	Male	Female
Total population	Children	1032	459	573
Number examined	Children	959	430	529
Number of cases	Children	40	22	18
Number of cases	Adults	43	28	15
Percentage of population examined		93·3 %		
Gross incidence		30·86 per 1000		
Child incidence		41·71 per 1000		
Sex incidence	Male	38·55 per 1000		
Sex incidence	Female	23·70 per 1000		
Sex rate		60·24%		
Child rate		48·19%		
Open case rate		15·66%		

Tables V and VI give a further analysis on the basis of epidemiological data.

TABLE V.

District Chingleput	Taluk Saidapet	Village Area 'C'		
Total population		Cases detected		Incidence per 1000
		Neural	Lepromatous	Total
1297	Males	37	13	50
1392	Females	31	2	33
Total		68	15	83

TABLE VI.

Analysis of cases by type and age.

Age group	Cases				Total for age group	Percentage of total cases.
	Neural		Lepromatous			
	No.	Percentage	No.	Percentage		
0–14	36	90·0%	4	10·0%	40	48·2%
15–34	17	85·0%	3	15·0%	20	24·1%
Over 35	15	65·2%	8	34·8%	23	27·7%
Total	68	81·9%	15	18·1%	83	100·0%

It will be noted that the gross incidence in this area is considerably lower than the incidence in the areas in which the main occupation of the population are weavers. The explanation probably lies in the fact that the weaving population owing to the method of working come into much closer contact with one

another than those who are occupied in other trades. Our work continues to emphasise the great importance of contact in the epidemiology of leprosy.

The leprosy preventive unit at Polambakam has continued its policy of night segregation and a comparison of the incidence of leprosy in the various villages in which night segregation has been applied during the past five years with figures for the two villages where no such segregation has been enforced is interesting.

TABLE VII.

Summary of surveys for the years 1939-45 in the villages connected with Rural Leprosy Preventive Unit.

	1939			1942			1945		
	Gross incidence	Open case rate	Child rate	Gross incidence	Open case rate	Child rate	Gross incidence	Open case rate	Child case rate
*Polambakam village	42·52	25·80	32·60	52·89	23·25	25·58	44·13	20·87	9·37
*Polambakam Cheri	45·55	23·80	19·05	44·00	13·63	13·63	38·79	16·66	16·66
*Perambakam village	38·46	28·57	14·28	38·64	31·25	18·75	30·95	15·38	15·38
*Perambakam Cheri	78·12	30·00	50·00	60·60	37·50	50·00	67·16	22·22	22·22
Maluvankaranai village	33·12	45·45	18·18	48·41	29·41	23·53	70·65	19·23	23·08
Maluvankaranai Cheri	29·12	50·00	50·00	56·33	30·77	38·46	49·79	25·00	25·00

*Night segregation enforced.

It will be noted that there has been a general reduction of the incidence of leprosy in three out of the four villages in which night segregation has been enforced. The only exception is Polambakam village where the incidence has apparently increased slightly. It is of interest to note that Polambakam village only began to take night segregation measures seriously after the year 1944. A further point of interest is the marked drop in the child rate in Polambakam village between 1939 and 1945. It will be noted that the open case rate has decreased while the gross incidence has increased, this may be due to the fact that some open cases have died and others left the village and the time interval may not be long enough for fresh open cases to have developed. The open case rate has decreased in all four villages during the past five years, in two of them very markedly. On the other hand in the two villages not under night segregation the incidence of leprosy in one of them is more than doubled, and in the other more than 1½ times the incidence in 1939. Both these villages have shown a steady increase in the incidence of the disease during the past five years. The open case rate has also decreased in these two villages owing to the death of a number of open cases and again the time interval for the development of fresh open cases may not as yet be sufficient. While no definite conclusions can be drawn, we feel that the overall picture shows a downward trend indicating a possible favourable turn in the epidemic of leprosy in these villages and encourages the pursuance of this experiment.

Therapeutic experiment:—The therapeutic experiments have proceeded at the Lady Willingdon Leprosy Sanatorium. The experiment on intradermal and subcutaneous injections have continued to confirm our previous opinion as shown by table VIII that the most efficient method of treatment is a combination of intradermal and subcutaneous injections.

TABLE VIII.

Intradermal group.

Negative	Much improved	Improved	Stationary	Worse	Total
20 (19·1%)	13 (12·4%)	29 (27·6%)	36 (34·3%)	7 (6·6%)	105 (100%)
		Subcutaneous & Intradermal group.			
31 (28·5%)	10 (8·2%)	48 (39·3%)	30 (24·5%)	3 (2·5%)	122 (100%)
		Subcutaneous group.			
2 (3·7%)	1 (1·9%)	15 (27·8%)	24 (44·4%)	12 (22·2%)	54 (100%)

Work has been done in an attempt to give high dosages of Ethyl Esters intradermally up to 30 CCS a week. There is evidence that if patients are willing to submit to this rigorous course of treatment, good results are forthcoming, but too few cases have so far been treated to form a comparison with other methods of treatment. Other experiments on treatment are being undertaken. At present no conclusions can be drawn.

Clinical and pathological:—The correlation of the clinical and histopathological findings is being continued. It is this section of the work that has suffered in the transference of the officer-in-charge of the enquiry from Chingleput to Vellore. The large group of so called intermediate cases continues to afford much material for study and from the study of further biopsy material previous opinion has been confirmed that the intermediate group of cases tend to end most frequently as grossly mutilated cases and it is the exception for such to turn into frank leuproma. Their ultimate prognosis however seems to be serious.

Animal experiments:—Animal inoculations have been continued and it is hoped to publish a paper shortly summarising six years' work.

PLAGUE

25. Plague Researches under the Director, Haffkine Institute, Bombay.

1. Casein hydrolysate from plague vaccine.

A. *Preparation of casein hydrolysate in large quantities:*—To produce vaccines in large quantities casein hydrolysate requires to be produced in batches of 200 gallons at a time. For this purpose a Pfaudler glass enamelled kettle with agitator rotating at 120 revolutions a minute and steam heated at 101°C was used. It was soon found that with this efficient stirring 72 hours period of digestion indicated by Mueller and Johnson for the preparation of the medium in five litre flasks was excessive. Further it was found that the medium prepared according to the instructions of Mueller and Johnson though gave a negative biuret reaction, still gave protein reaction on intradermal injection. A great deal of work was done to work out the exact conditions under which this medium could be produced in large batches. The work is now completed and details will be published shortly in a paper.

B. *Optimum duration of growth of Pasteurella pestis in the liquid medium :*—The same batches of casein hydrolysate dispensed in one litre quantities in modified Haffkine flasks were sown with the same culture of *Pasteurella pestis*. They were incubated at 28°C; some for one week, others for 2 weeks and a third lot for 4 weeks. These growths were sterilised by the addition of 0·1 per cent formaline and preserved by the addition of 1·0 mg. of phenyl mercuric nitrate per 100 c.c. of growth. The following mouse protective values were obtained :—

	Mouse protective dose.
One week's growth	0·009 c.c.
Two weeks' growth	0·004 c.c.
Four weeks' growth	0·004 c.c.

C. *Keeping qualities of casein hydrolysate plague vaccine :*—A tested batch of vaccine was stored at 0°C and at 37°C. The mouse protection value of the samples was determined from time to time. A standard agar-grown vaccine was stored and tested the same way. The following results were obtained :—

	Casein Hydrolysate Vaccine. Mouse protection dose.		Agar Vaccine. Mouse protection dose.	
	Stored at 0°C	Stored at 37°C	Stored at 0°C	Stored at 37°C
Fresh vaccine ..	0·003 c.c.	..	0·004 c.c.	..
After 2 months' storage ..	0·003 c.c.	0·003 c.c.	0·004 c.c.	0·007 c.c.
After 4 months' storage ..	0·004 c.c.	0·004 c.c.	0·004 c.c.	0·006 c.c.
After 8 months' storage ..	0·004 c.c.	0·004 c.c.	0·004 c.c.	0·006 c.c.

2. The pulicidal value of D.D.T. and 666.

Experiments on determining the pulicidal value of dichlordiphenyl-trichlorethane, popularly known as DDT, and of gamma isomer of benzene hexachloride (gammexame), generally known as 666, were carried out. Both were used in the form of sprays and dusts. Two types of experiments with

each of the above mentioned synthetic organic compounds were conducted; one in experimental jars, and the other in artificial, W-shaped, twenty feet long burrows simulating a natural rat burrow.

For experiments with spraying, saturated solutions of DDT and 666 in kerosene oil and kerosene soap emulsions containing both these compounds, were used. Only experiments in jars were tried with sprays. Each jar containing 1000 grammes of sand, was at first sprayed with a measured quantity of solution or a diluted emulsion, and then shaken in such a way that its entire quantity was thoroughly mixed with the sand. About 0·1 gramme of DDT in solution with kerosene oil gave 100 per cent kill of adults of the Indian rat-fleas in the experiments tried up to 120 days after spraying. Even 0·021 gramme of DDT in the form of kerosene soap emulsion killed 100 per cent rat-fleas, so far, up to 49 days. Similarly 0·0044 gramme of 666 in the form of kerosene soap emulsion killed 100 per cent fleas in the experiments tried up to 28 days.

Delousing powder, 10 per cent DDT in pyrophyllite, U.S. Patent No. 23229074, and a powder containing 20 per cent 666, were used for experiments with dusting. Both jars and burrow experiments were tried with dusts. In jar experiments 0·1 gramme of DDT, when mixed with 1000 grammes of sand, gave 100 per cent kill of rat fleas in the experiments tried up to 103 days after dusting, and 0·2 gramme of 666 up to 48 days. In artificial rat burrow experiments, due to abruptly diminishing distribution of dust in the first arm and gradually diminishing in the others, when blown into a W-shaped burrow by a Cyanogas " A " dust foot pump duster, the exact quantity of dust at different places could not be determined, but only a quantity necessary to disperse the dust up to the farthest end of the burrow was ascertained. A considerable accumulation of the dust in the first arm of the W-shaped, artificial burrow occurred. This is bound to happen even in a natural rat burrow, which will undoubtedly prove beneficial from the view point of plague control, as fleas leaving a rat burrow will get covered with the dust and thus would get killed subsequently. A hundred per cent kill of rat fleas was obtained even at the farthest end of the burrow for a number of days after dusting; but this could not be achieved in every case. Dusting of an artificial burrow with 65 grammes of 10 per cent DDT in pyrophyllite, gave 90 per cent kill of adult rat fleas in an experiment tried up to 135 days, after dusting at a point about 14 feet away from the point of blowing. Similarly, when dusted with 50 grammes of 20 per cent powder of 666, about 100 per cent of adult fleas were killed off after 65 days dusting at a place about 9½ feet away from the entrance of the burrow. Beyond these points in both cases the mortality rate was variable and lower. The burrow experiments were done under undisturbed conditions of the burrow soil without a rat, in order to determine any deterioration of DDT or 666, when in contact with the moistened conditions of the soil for a long time.

This work is being done by Dr. Sharif of the Department of Entomology of the Haffkine Institute, without a grant from the I.R.F.A.

26. Plague enquiry in the Salem District under the Director of Public Health, Madras.

The plague enquiry at Hosur comprised of the studies of the prevailing rodents and their fleas contributing to the endemic plague of the area and intensive application of cyanogas in plague control.

2. Routine cyanogas fumigation of rat holes, and other rat harbourages was carried out in all the villages in the experimental area as usual. In villages where cyanogas was applied soon after the detection of rat plague, the infection was aborted and no further rat falls or human cases occurred thereafter. In other villages where control measures were adopted after the report of human cases, the epidemics either stopped or were very mild.

3. A survey of fleas on R. rattus revealed the following :—

The general flea index ranged between 1·38 in April to 5·7 in February 1945. The X. astia index is below one per rat throughout the year. It rises to 0·9 during June, while it is as low as 0·04 in April and its seasonal fluctuations do not follow either the trend of plague or those of the other fleas. The cheopis index varies between 0·6 in April to 3·32 in August ; the brasiliensis index varies between 0·6 in May to 3·65 in February. It is interesting to note that the coolest season of Hosur (October to February) is more congenial to the prevalence of X. brasiliensis than X. cheopis. Of both these efficient vectors, the former flourish best in those months during which most of the plague in the area also occurs.

4. 835 field rodents and their fleas were examined during the year for studies in sylvatic plague. Over 96% of the fleas were X. astia. Last year three infected gerbils were reported from Mathagiri village which had human plague at that time. This year 7 dead gerbils picked up within a furlong from three different sources of Edayanattur, Mathagiri and Dimmur during the months of March, April and August respectively were positive for plague. There was no plague either in these villages or in the neighbourhood during these months. These observations indicate the existence of sylvatic plague among rodents of Hosur even when infection was not apparent among domestic rodents.

5. Examination of rodent nests was continued to study the bionomics of the various fleas in respect of climate, hosts and habitats. It has observed as before that the predominant fleas on R. rattus which are chiefly concerned with the spread of bubonic plague in this area are X. brasiliensis and X. cheopis, the former predominating during the plague months.

CLINICAL RESEARCH

27. Clinical Research Unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The Clinical Research Unit started functioning from the 1st July, 1945. The following brief statement refers therefore to the work carried out in the last five months.

State of saturation with Nicotinic Acid and Riboflavin in Oral Cancer Patients:—Previous clinical and laboratory work at this institution had suggested that many oral cancer patients showed evidence of deficient vitamin B complex utilisation. Careful excretion studies of nicotinic acid and riboflavin after administration of test doses were carried out on (a) eight patients with oral cancer, (b) eight persons showing a deficient state of vitamin B nutrition without any evident cancer and (c) eight healthy laboratory workers. The results of these studies are very suggestive and are tabulated below :

TABLE I.

*Average excretion of total nicotinic acid in urine**

	Days.†				
	i	ii	iii	iv	v
Normals	44·8	74·5	80·3	57·0	48·7
‡Intermediate group ..	30·2	55·3	63·6	39·3	36·0
Cancer	35·9	52·0	57·2	44·7	37·5
*Average excretion of total riboflavin in urine**					
Normals	1·0	1·03	0·97	1·74	2·21
‡Intermediate group ..	0·63	0·61	0·61	1·16	1·50
Cancer	0·76	0·76	0·72	1·26	1·62

*Each group consists of eight persons.

†Test doses of 250 mgms. of nicotinamide were administered after the 24 hour urine collection of 1st and 2nd days; and 12 mgms. of riboflavin after the 3rd and 4th days. The total nicotinic acid comprises of nicotinic acid and trigonelline.

‡Intermediate group consists of persons showing a degenerative and inflammatory condition of oral mucosa suggestive of early vitamin B deficiency.

TABLE II.

	Average amount of nicotinic acid excreted in urine during 24 hours.	Average extra amount of nicotinic acid excreted in urine during 24 hours after a dose of 250 mgms. of nicotinamide.
Normals	50·3 mgms.	26·8 mgms.
Oral Cancer patients ..	39·0 mgms.	15·6 mgms.

TABLE II. (*Contd.*)

	Average amount of riboflavin excreted in urine during 24 hours.	Average extra amount of riboflavin excreted in urine during 24 hours, after a dose of 12 mgms. of riboflavin.
Normals	0·98 mgms.	0·97 mgms.
Oral Cancer patients ..	0·76 mgms.	0·68 mgms.

These figures have been analysed statistically and show significant difference.

It is now intended to extend this work after administration of test doses of thiamine, nicotinic acid and riboflavin simultaneously on the lines suggested by the work of Keys and others.

1. Keys & others : Am. Jour. Physiol. 144 : 5 : 1945.
2. Keys & others : Jour. Nutrition. 27 : 165 & 485 : 1944.

28. Enquiry on Naga sore under Dr. S. R. Pandit at the Pasteur Institute, Shillong.

During the period under report, nineteen cases of naga sore were admitted to the Clinical Research Hospital attached to the Pasteur Institute and were kept under observation for varying periods. Nine of these cases were from Lakhimpur District, six from the Khasi Hills District, two from Kamrup, one from Nowgong and one from Madras. All the sores were in the active phase. Nine of the patients had a single sore whereas in the remaining the sores were multiple ranging from two to six. In all of them the sores were distributed over the lower extremities below the knee. Thirteen gave a history of cut or injury as having initiated the sore ; in the rest the sore originated spontaneously as a vesicle or bleb. There was evidence of malaria (positive blood finding and/or enlarged spleen) in nine patients. Ten of the cases were negative to helminthic infections ; of the remaining nine, three had ankylostomes, five other helminths and one giardial infection. As regards the state of nutrition, one had *xerosis opthalmia* accompanied by night blindness and nearly half of the remaining individuals suffered from a mild to a moderate degree of anaemia due to malaria and other causes.

Microscopic findings.—Repeated examinations of smears stained by different stains and by dark ground illumination were made in each case. Such examinations were carried out from day to day in a few selected cases. The results may be summarised as follows.

A constant finding was fusiform bacilli. These were few or occasionally present in healing ulcers but invariably preponderated in early sores. Spirochaetes were seen in only five cases, four of which came from the Khasi Hills and one from Madras. Gram positive cocci were found in all the cases and in a few there was an occasional fungus. No corynebacterium or acid-fast organism was present in any of the cases. A gram positive slender curved bacillus was met with in twelve cases.

Cultural investigations.—The fusiform bacillus from its constant presence and predominance in every sore seemed to play an aetiological role. Considerable time was therefore devoted to obtaining pure cultures of the organism. Difficulty was experienced initially; primary growth was very poor, if at all, and sub-cultures failed. Finally, after many attempts, pure cultures of fusiformis were obtained from two cases, the medium used being 20% sheep blood agar with or without gentian violet for inhibiting some of the other contaminants. Successful cultures were obtained in an atmosphere of hydrogen with 5 to 10% carbon dioxide. So far as the workers' experience goes, it was necessary to have fresh media not more than 24-48 hours old. It was found possible to make the organism grow on ordinary agar after two or three sub-cultures on the blood medium. The organism is a strict anaerobe.

Among the earlier attempts to cultivate the organism the following special media were tried, besides routine media: Slanetz and Rettger's potato extract agar with varying concentrations of gentian voilet, Smith's semi-solid medium, and liquid media with different reducing substances.

A detailed study of the biochemical characteristics of the organism etc. is being pursued and will duly be reported.

Pure cultures of the slender gram negative bacillus referred to above were obtained without much difficulty. The organism requires animal protein for its primary growth. It is a motile, monotrichate organism utilising none of the sugars. Agglutination tests were put up with sera of ten of the patients in whom this organism was found. Four were positive, the titres being 40 in one and 20 in three cases. The rest were negative. Six sera received at this Institute for routine serological tests were all negative.

Experiments on transmission.—Attempts were made to transmit the disease in animals by inoculation by various routes of a fresh suspension in broth of the discharge from sores. They were all unsuccessful. The animals so far used were the rhesus monkey, guinea pig, white mouse, rabbit and white rat. Intracutaneous inoculation on the other hand, into a human volunteer resulted in the formation of a vesicle at the site of inoculation which later assumed the typical characters of the sore with fusiform bacilli predominating. Subcutaneous inoculation into the same individual resulted in a diffuse slight swelling which later subsided completely.

A suspension of the fusiformis culture (fifth sub-culture after the primary isolation) was inoculated intracutaneously into a human volunteer. No ulcer resulted but the work is being continued. The same suspension was also injected intracutaneously into animals (listed earlier) but with negative results. This is however not surprising in view of the earlier findings with fresh pus, which suggested the refractory nature of the animals to naga sore.

Epidemiological study.—A detailed history of 108 cases was taken. Seventy eight of these cases were from tea estates in the Dibrugarh area and the rest from the Sadiya and Phasighat frontier tract. The findings are summarised in the following lines.

It is a disease of adolescents and young adults; 62% of the cases were in the age group 10-30 and 42% in the age group 20-30. Only 2% were below 10 years. 58% of the cases were of the female sex. The ulcers were confined to the lower extremities below the knee. In 66% the ulcers were single and in 34% multiple; where multiple, there was evidence of auto-inoculation. In 66% of the cases there was a history of spontaneous appearance of a vesicle but

in the rest there was a history of trauma, leech bite or insect bite. There was no evidence that an insect vector caused the disease. There was definite seasonal incidence, the great majority of cases occurring in the period July to September, which coincides with the rains. Two adjoining estates were sometimes found having different histories, one escaping altogether during the year, while there were many cases in the other. As regards occupation, the incidence was very low among plantation labourers engaged in indoor work in the factories, but much higher in those engaged in outdoor work or in hill tribes working in the jungles.

As regards immunity, one attack did not seem to prevent a subsequent one. 8% of the cases had recurrence within a year, in one of whom a second sore developed three months after the first had healed completely.

Nutritional deficiency.—Sample dieteries of the plantation labourers were grossly deficient in vitamins and essential proteins. The diets of tribesmen of the frontier were not so. Still the two groups suffered to an equal extent during the outbreak of 1942. That dietetic factors played little part in the aetiology was also suggested by the fact that in the series examined, there were comparatively fewer people in the older age groups—precisely the groups in whom malnutrition was more evident.

29. Enquiry into hypoproteinaemia and other conditions associated with malnutrition under Dr. John Lowe at the School of Tropical Medicine, Calcutta.

This enquiry, sanctioned in March this year, could not be instituted until September owing mainly to the illness of the officer in charge. Some preliminary work had, however, been done before this, which is here summarised with the work now in progress.

A study of simple methods of blood protein estimation. From the Medical Research Council in England details were received of the methods which they have been studying, particularly the method of Phillips, Van Slyke *et al*, which is an extremely simple method of estimating the *total blood proteins* by a series of copper sulphate specific gravity bottles. On this method the Medical Research Council reported very favourably.

This method has been adopted and studied here. Total blood protein estimations by this method have been carried out in about 150 cases. Certain technical difficulties were encountered and had to be overcome. The results now being obtained appear to be fairly accurate and of considerable value. Dozens of tests can be done in a few hours.

The work done, however, revealed the fact that the value of total protein estimations is often rather limited. In some cases a normal total protein will be associated with oedema caused by hypoproteinaemia because the more important albumin fraction is low while the globulin fraction is high. Without the estimation of the albumin and globulin fractions, any work done is of limited value. The Kjeldahl method requires a well equipped laboratory and is of limited application. Professor King of the Post Graduate Medical School, London, has suggested for the use of this enquiry *the biuret method of estimating blood protein fractions*. This method is now being studied. It is relatively

simple and several tests can be done in a few hours with a minimum of equipment. The accuracy of this method is now being tested by comparison with the Kjeldahl method in the same blood. Results so far obtained indicate that the method is very useful for clinical purposes though perhaps less accurate than other methods which, however, demand much more time and apparatus.

Preliminary studies of different methods of *treating hypoproteinaemia* have been made in about twenty patients. Despeciated bovine serum prepared by the method of Edwards (1944) tended to produce allergic reaction, especially if second injections had to be given. The intravenous administration of reconstituted human blood plasma at double strength is useful in reducing nutritional oedema by increasing osmosis, but its actual nutritional value is limited. Intravenous injection of amino-acids is useful, but no preparation is free from dangers of severe reaction. The oral administration of a high protein diet is much the best general method of treatment. Mercurial diuretics are of value in combating *the marked oedema* of severe hypoproteinaemia.

Results so far obtained indicate that the copper sulphate method of estimating total proteins and the biuret method of estimating protein fractions should be of wide applicability and utility in India. It is proposed to carry out these estimations in three groups of people: (i) normal, healthy, well-nourished Indians, (ii) unselected people in Indian villages, (iii) cases of disease admitted to the Carmichael Hospital for Tropical Diseases. Each group will be made as large as possible to make the findings of statistical significance; 100 normals, and 100 or preferably 200 in the other two groups will, if possible, be studied.

30. Enquiry on hill diarrhoea under Dr. G. Panja at the School of Tropical Medicine, Calcutta.

A field investigation was conducted at Darjeeling in June and July. Typical cases of hill diarrhoea as described in the Encyclopoedia of Medical Practice, characterised by early morning diarrhoea, presence of copious, pale yellow, liquid stools and borborygmi and absence of fever, abdominal pain and cellular exudate in the stools were not met with. Cases of atypical diarrhoea, frank dysentery and indigestion were seen. The chief object of the investigation was to find out whether the disease was due to any infection of the bowels and hence great stress was laid on the cultural examination of fresh stools on several new and successful media, such as Difco SS agar, D.E.C. medium (Panja & Ghose), desoxycholate citrate agar (Leifson).

Examination of stools.

201 samples of faeces from 165 cases were examined. Capt. Rayson of the U.S.A. military rest camp at Jalapahar was very kind to bring a large number of samples from his cases. The majority of the samples were from cases with some sort of bowel complaints. Stools were classified as formed, semi-formed, liquid, mucoid, muco-purulent and mixed with blood.

Paracolon organisms, Newcastle bacillus and Flexner's bacillus were commonly isolated.

Several strains of apparently Flexner's bacillus inagglutinable with the nine stock Flexner sera were isolated. In one outsider (Mrs. D) suffering from copious diarrhoea occurring every time she came to Darjeeling, an inagglutinable Flexner's bacillus was isolated several times. In another case (Ck) suffering from violent diarrhoea *Bact. Flexneri IV* was recovered. In another outsider (Miss S), a paracolon organism was isolated. In several cases, a very interesting new paracolon organism was isolated. This is a motile late lactose-fermenter, producing acid only in lactose but acid and gas in glucose, manitol and maltose and is agglutinable with Schmitz bacillus serum. Injected intravenously in small dosage, it kills a rabbit within 24 hours, and is recovered in almost pure culture from the ileum which is congested and contains a glairy mucoid fluid. It produced a mild temporary diarrhoea when given by mouth to a volunteer and was recovered from his stool. An agglutinin was found in one of the patient's serum (Miss R) for Schmitz's bacillus but no other dysentery or coliform organisms. A cross absorption test was done with Schmitz's bacillus. The test showed that the organism was antigenically related. In one case (Baby R) suffering from copious purging and stool showing cellular exudate and red blood cells, *Bact. pseudo-carolinus* was isolated. In many cases of atypical diarrhoea with or without mucus in the stool, no pathogenic micro-organism was found. This suggests the idea that some cases of diarrhoea may be due to causes other than an infection, as will be shown later.

Cross absorption test with the new organism.

Sera	Cultures		
	Schmitz	New bact.(H)	New bact.(O)
Schmitz	1600	-100	+ 100
Sch. Serum + Sch. bact. ..	..	..	..
Sch. Serum + New bact. ..	1600	..	..
New bact.	1600	12,800	6400
New bact. Serum + New bact. ..	..	..	..
New bact. Serum + Sch. bact. ..	..	12,800	3200

The above table shows that the new bacterium is antigenically related to Schmitz's bacillus.

Helminthic infection with Ascaris lumbricoides and hookworms was very frequent. Entamoeba histolytica infection was found much less frequent than the bacterial infection. Yeast infection was rare.

Biochemical examination of stools.

This was done with a view to find out whether physical conditions such as high altitude (Barometric pressure at Darjeeling 22·906) humidity (95-100%) and lowered hill temperature (52-70°F.) exerted any effect on the gastro-intestinal secretions. In diarrhoeic stools, amylase and trypsinase were found considerably reduced as compared with normal stools. Lipase was absent in all normal and abnormal stools excepting in one only of the latter group. In the plains such a finding is absent in diarrhoeic stools, whereas in normal stools, a similarity exists. For example, amylase varied from 20 to 100 and trypsinase from 50 to 140 in normal stools at Darjeeling whereas in diarrhoeic stools, amylase varied from 0·6 to 10 and trypsinase from 2 to 20 only. This suggests that some cases of diarrhoea in which no bacterial infection is discovered may be due to physical conditions affecting the gastro-intestinal secretions.

Examination of water supply.

The water filtration plant and chlorination of water at Ghoom was inspected and found working satisfactorily. Filtered and chlorinated water was being supplied to the people for the last 3 years. Chlorination was being done at 0·6 per mille during the rainy season and 0·3 to 0·4 during the rest of the year. Tap water collected and tested for chlorine showed positive reaction.

An analysis of water from the Senchal lake, the source of water supply for Darjeeling was carried out on 2-7-45 during the monsoon period. The water was found quite satisfactory in as far as the chemical constituents were concerned, the total solids being only 8·421 per 100,000; nitrates, iron and sulphates were absent.

Incidence of disease at the Victoria Hospital, Darjeeling.

The figures collected at the Victoria Hospital, Darjeeling show a slight decrease in the incidence of diarrhoea and dysentery during the last 3 years during which chlorination of water supply was done.

31. Enquiry into erythroblastosis foetalis and its association with the Rh factor under Lt.-Col. S. D. S. Greval, I. M. S., Imperial Serologist to the Government of India at the School of Tropical Medicine, Calcutta.

I. Determination of percentage of Rh +/— state of pregnant women attending the antenatal clinic of the Eden Hospital, Calcutta.

II. Intragroup reactions and other anomalous reactions in the bloods of pregnant women from the Eden Hospital.

III. Percentage of Rh +/— state of unselected cases from samples of blood received in the Imperial Serologist's Laboratory from different hospitals for other tests.

IV. Study of anti-Rh animal sera prepared in rabbits and guineapigs.

V. Comparison of tube and slide method in haemagglutination for Rh study.

VI. Study of suspected families.

I. Pregnant women (period of gestation 5 to 9 months) attending the antenatal clinic of the Eden Hospital were selected. Obstetric history as regards previous pregnancy including abortion, miscarriage, malformation and anaemia or jaundice of previous issues and history of previous blood transfusions, if any, suggesting Rh sensitization were recorded. Bloods were taken both for rbc and sera. Cells were tested for Rh +/— state with animal anti-Rh serum prepared in this laboratory. Both tubes and slides were used. Women of various communities have been tested with the following results :—

	Tube Method.				Slide Method.			
	Indians.		Others.		Indians.		Others.	
	Nos.	%	Nos.	%	Nos.	%	Nos.	%
Positive =	145	84·80	54	87·10	146	85·39	52	83·88
Doubtful =	13	7·60	nil	nil	12	7·01	2	3·22
Negative =	13	7·60	8	12·90	13	7·60	8	12·90
Total =	171		62		171		62	

II. Sera from bloods of pregnant women taken 2 hours previously (complement being active) were tested for intragroup reaction to detect any anti-Rh or anti-Hr bodies, if present. Each serum was tested against compatible cells of various groups taking 3 samples of cells from different sources of each group, e.g.

Serum a from subject of group B tested against 3 different samples of rbc of group O & B.

Serum b from subject of group A tested against 3 different samples of rbc of group O & A.

Serum ab from subject of group O tested against 3 different samples of rbc of group O only.

Serum o from subject of group AB tested against 3 different samples of rbc of group O, A & B.

Method.—3 drops (1 drop = 1/50 c.c.) of the serum and cell suspension were mixed in a tube. Both agglutination and lysis were looked for, agglutination by crenation and non-fluidity of the edge of the deposit and lysis by coloration in the supernat. (i) Presence of crenation, however, slight and non-fluidity of the edge or/and (ii) presence of lysis would determine the positive reaction, i.e. the presence of antibody in the serum. Results tabulated as follows :—

Total cases done = 318.

Positive reactions :—

1 only in a subject of group O, Rh±with group O cells from 3 subjects.
1 only in a subject of group B, Rh+with group O cells from 3 subjects.
1 only in a subject of group O, Rh—with group O cells from 41 subjects including two known Rh—cells.

Obviously, these anomalous reactions were not due to anti-Rh body in the human serum.

III. Unselected samples of *Indian* blood received for other tests were used for the purpose. In this lot citrated samples were not available. Rbc were obtained from clots by washing. Results tabulated as follows :—

	Tube Method.		Slide method.	
	Nos.	%	Nos.	%
Positive =	245	87·51	247	88·58
Doubtful =	17	6·07	11	3·92
Negative =	18	6·42	21	7·50
Total	280	..	280	..

IV. Both rabbits and guineapigs were used. Rabbits were found more satisfactory as a rabbit yielded more serum which was divided into many lots and could be used for several months, if found satisfactory by trial on 100 cases. Later, other sera were found to be satisfactory or otherwise in comparison with this serum. The dilution of the serum suitable for the test was determined by trial and error. Mostly 10 times the minimal agglutinating dose for the monkey rbc sufficed for the detection of Rh positivity of human rbc.

The highest titre obtained for the monkey cell was 1 in 2000 and therefore for human subjects 1 in 200. Each sample of blood was tested with 2 dilutions of this anti-Rh serum, i.e. 1 in 100 and 1 in 200. A negative reaction with 1 in 200 was considered sufficient.

V. *Tube method.* For Rh determination 5 drops each of 2% rbc suspension to be tested and the anti-Rh rabbit immune serum delivered from a calibrated finger teat pipette (50 drops to a cc) are mixed in a 3″ × ½″ tube. The tube is left at blood heat for 2 hours.

Slide method. A slide is divided into 3 compartments. First two compartments contain equal volumes of rbc and two strengths of testing serum. Third compartment is a control of rbc with the dilution of normal rabbit serum corresponding to the weaker strength of the testing serum. Mixing is thorough. The slide is examined after 1 hour (in a moist chamber).

Tube vs. slide method. There was not a single blood that gave positive by one method and negative by the other method out of a total of 513 bloods tested. But negative by one method gave doubtful by the other and vice versa and also some doubtful by one method gave positive by the other and vice versa. 6 doubtful cases corresponded by tube and slide method in the Eden Hospital series and 9 cases corresponded in the other series. These results are tabulated as follows :—

Grades	Eden Hosp. cases	Other cases
T + S ±	5	7
T ± S —	5	7
T ± S +	5	7
T — S ±	5	7
T ± S ±	6	9

(T = Tube method. S = Slide method.)

A suggestion emerges that for the purpose of transfusion an Rh ± donor should be taken as positive and an Rh ± recipient should be taken as negative.

VI. Five families were studied including three children. In two cases mothers were negative of which one had antibody formation which evidently was not an anti-Rh body as it agglutinated 41 different cells of its own group including 2 known Rh-cells. In one case mother was Rh ±. All the fathers and children were Rh +. The study of families has been facilitated by contact with the local lady medical practitioners and Red Cross Organisation.

32. Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

The first three months of the year were occupied in completing the study of ringworm of the body to one thousand cases—to determine the species of ringworm fungus common in India—this was in continuation of the last year's work.

The experimental and clinical study of some drugs suitable for use as a prophylactic measure against ringworm infection was also completed. The results of the laboratory experiments were published in the last year's report. In that report it was mentioned that glacial acetic acid was found to be a suitable drug and a two per cent solution of it in 25 per cent alcohol was effective in preventing ringworm infection. Clinical trial however proved that a two per cent solution is effective in preventing ringworm infection in persons who never had the disease. For a susceptible person, and to prevent a re-infection or a relapse a 4 per cent solution in 25 per cent alcohol is required. The 4 per cent solution is non-irritating, non-corrosive and fully effective. The paper is ready for publication.

The main work in the current year consisted in the culture of the fungus *Malassezia ovalis* and preparation of an antigen for the complement fixation test in cases of Seborrhoeic dermatitis.

Seborrhoea and seborrhoeic dermatitis is a big problem in dermatology. The fungus *Malassezia ovalis* is said to be one of the principal factors concerned in this condition. More than eight per cent of cases seen in the skin out-patient department in Calcutta are diagnosed as such. Yet there are no means at present to confirm the diagnosis made clinically in such a large number of dermatitis.

The possibility of a positive antigenic response of the blood serum from seborrhoeic cases to an antigen prepared from the fungus *Malassezia ovalis* was thought of and the preliminary work done in previous years being encouraging the work was taken up this year.

A. Culture of the fungus.

Culture and isolation of the fungus Malassezia is difficult. Some of the methods described in the literature (available here) were tried but none of them were entirely satisfactory. After several attempts an improved method has been found to give better results and this is being tried now. Some of the older methods tried gave the following results:

(1) Modified Petroff's media with the addition of 0·1 per cent gentian violet—scales inoculated in the dry upper portion of the media—

Number of cultures put up	.. 81
Number of positive cultures	.. 13
Isolation in pure form	.. 4

(2) Emmons's liquid media—It is a liquid acid dextrose broth media to which 23 to 44 per cent of glycerine is added. In this the growth of the fungus was easy and satisfactory but the secondary contaminating organisms were so profuse as to make the isolation almost impossible.

(3) New media with cod liver oil—(Details are being prepared for publication).

Number of cultures put up	.. 12
Number of positive cultures	.. 8
Number of isolation in pure form	.. 5

So there are at present eight cultures of *Malassezia ovalis* from eight different cases.

B. Preparation of antigen.

I. Kolmer's method for preparing antigen from fungus:—It is a suspension of the organisms in phenolised distilled water. Four lots of the antigen have been prepared by this method—the results are as follows:

Lot No. I.—Titration anticomplementary upto 1/6th dilution.
Fixation test was done with 1/8th dilution.

		Positive reaction	Negative
Number of clinical seborrhoeic cases tested	43	31	12
Number of controls—	20	4	16

Lot No. II.—Titration upto 1/2 dilution—anticomplementary upto 1/3 and 1/4th dilution gave lytic reaction.

Test was done with 1/6th, 1/8th and 1/12th dilutions.

85 cases were done but the results were very conflicting and so this lot was given up.

Lot No. III.—Upto 1/2 dilution not anticomplementary.
Test was done with 1/2 dilution.

		Positive reaction	Negative
Number of cases of clinical suborrhoea tested	63	40	23
Number of controls—	20	5	15

Lot No. IV.—A polyvalent antigen prepared from 3 cultures of *M. ovalis* (from three different cases) and one of *M. furfur* from a case of Pityriasis versicolor.

Titration anticomplementary upto 1/12th dilution.
Fixation test was done with 1/16th dilution.

No. of cases— 28; Positive reaction 1; Negative 27.

This lot has been given up.

II. Antigen prepared from dried powder of the fungus grown in liquid media. Two lots were prepared.

Lot No. I.—Anticomplementary upto 1/8th dilution.
Complement fixation was done with 1/12th dilution.

		Positive reaction	Negative
Number of cases tested—	31	14	17

Lot No. II.—Anticomplementary upto 1/3rd dilution.
Complement-fixation test was done with 1/5th dilution.

		Positive reaction	Negative
Number of cases tested—	31	12	19

Other methods of preparing antigen and standardisation of the same are being tried.

PHARMACOLOGY

33. Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta.

1. *Rauwolfia serpentina.*—A resin freed from oily constituents has been found to be responsible for hypnotic action. In order to isolate the most potent fraction of the resin it was further fractionated into four portions viz., Alcohol, Ether, Chloroform and Ethyl Acetate soluble fractions. These were then separately administered to rats and all the fractions produced some hypnosis. It is therefore concluded that the whole of the resin is active. It has also been observed that the four different fractions obtained as above becomes on keeping, less soluble in the respective solvents.

2. *Daemia extensa.*—The active principle of *Daemia extensa* is of glucosidic nature and exists with toxic constituents. A large number of experiments have been done both with the crude and purified extract and the results have been communicated for publication. The drug has a marked stimulating action on the intestine, bladder and uterus where its action is better than on the pituitary. It raises the blood pressure and is well tolerated by laboratory animals. Attempts are being made to give it a clinical trial.

3. *Vitex peduncularis.*—An impure crystalline substance insoluble in alcohol was isolated and found to have some anti-hemolytic property in vitro. Further attempts to isolate the principle in quantity have so far been unsuccessful.

4. *Vanda roxburghii.*—An active constituent has been isolated which appears to be of glucosidic nature. It is a parasympathetic stimulant and stimulates all forms of involuntary muscles. A large number of experiments have been performed and the results will shortly be communicated for publication.

5. *Terminalia chebula and Terminalia bellerica.*—The presence of anthraquinone bodies in these two plants have been reported for the first time. Attempts have been made to correlate the purgative potency with the anthraquinone content (in experimental animals) but the presence of a large amount of tannin renders the results uncertain and difficult of interpretation. In human volunteer T., *chebula* produced excellent purgation but T. *bellerica* did not, though given in adequate doses on the basis of its anthraquinone content.

6. *Ipomoea aquatica.*—The following fractions have been prepared for pharmacological investigation. Petroleum ether extract, 1·64%, ether extract 0·67%, ethyl acetate extract 0·46%, chloroform extract 1·25%, alcohol extract 9.42%. The alcoholic extract contains a fair quantity of Kcl and a bitter principle which is insoluble in water.

7. *Argyreia speciosa.*—A total alkaloidal extract has been found to produce inconsistent results. This is due to deterioration of active principles. Various methods have been tried to prevent this deterioration without much success so far.

8. *Leea hirta.*—This plant has been found to contain an alkaloid whose pharmacological action is under investigation.

Chemotherapeutic studies in malaria and amoebiasis.—Various antimalarial remedies of indigenous origin, chiefly a combination of reputed bitter plants

having some antipyretic action, have been tried in outdoor and hospital cases but none of the drugs so far investigated has substantiated the claim made on their behalf by the manufacturers.

A sulphonamide derivative locally manufactured, has been tried in both avian and human malaria with encouraging results. Further trials are in progress and the results will shortly be communicated for publication.

10. *Enteroquinol*, a locally manufactured compound similar to Enteroviofform, has been found to be as effective as any foreign preparation.

11. *Diiodoquin*, another hydroxyquinoline derivative but containing 2 atoms of iodine, has been found to give encouraging results. Further work is in progress.

34. Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S.(R) at the Drug Research Laboratory, Jammu, Kashmir.

Chrysanthemum cinerarifolium vis.

1. Work on pyrethrum was continued and a number of samples of pyrethrum from eleven different plantations were analysed to see the effect of *altitude* and the *method of* drying on the pyrethrine content of the flower-heads.

(a) *Effect of altitude*:—Samples from plantations growing at different altitudes ranging from 5,000 to 8,000 ft. were analysed. The results of such analyses show that whereas pyrethrum can be profitably cultivated at 5,000 ft. an altitude from 6,000 ft. to 8,000 ft. gives the best results so far as the yield of pyrethrines is concerned.

(b) *Methods of drying*:—After harvesting, the flower-heads have to be dried. For studying this problem samples were dried under different conditions. It has been found that the samples dried partially in the sun and partially in the shade give highest yield whereas the samples dried throughout in the sun come next and the flowers dried throughout in the shade give the lowest yield of pyrethrines. Since the number of such samples received was small this point will be investigated in detail in the next crop.

2. *Extraction of pyrethrum flower-heads with different solvents.*—For preparation of concentrated extracts of pyrethrum, it is essential to use a solvent which removes pyrethrines completly and at the same time extracts less of inactive material. To study this problem, extracts were prepared with all the organic solvents. As a results of this study it was found that although petroleum ether gave best results still for commercial purposes Benzene would be most suitable.

Essential oil-bearing-plants.

A number of essential oil bearing plants grow wild in Kashmir. A preliminary investigation of some of these was done to study the percentage yield of the oil of the plants and their physical properties.

1. *Mentha arvensis* Linn. was taken up this year. A large quantity of the herb was steam-distilled and physical constants of the oil determined.

Yield of the oil	.. 0·45 per cent
Specific gravity	.. 0·0161 at 15°C.
Refractive index	.. 1·474 at 20°C.

On allowing the oil to stand at 0°C. no crystals of menthol separated.

2. *Thymus serphyllum* Linn. (Wild thyme; Vern. Ban ajwoin) N.O. Labiatae.—The plant is very common in Kashmir valley and is used in indigenous medicine in complaints of stomach and liver and as a remedy for toothache. The whole dried plant on steam-distillation yielded pale brown oil with a faint thymol like odour.

Yield of the oil	.. 0·73 per cent
Specific gravity	.. 0·9404 at 15°C.
Refractive index	.. 1·512 at 20°C.

3. *Nepeta ciliaris* Benth. (Vern. Zufa); N.O. Labiatae.—The plant is much used in the country medicine in fevers and coughs. It grows abundantly in Kashmir valley. For investigation the dry leaves and flower tops were steam-distilled and the oil obtained for studying the physical constants.

Yield of the oil	.. 0·54 per cent
Specific gravity	.. 1·061 at 15°C.
Refractive index	.. 1·499 at 20°C.

4. *Skimmia laureola* Hook. N.O. Rutaceae.—The plant is very common in the valley especially at Gulmarg and Pehalgam. It is used as an offering and the leaves and branches are burnt as an incense. The green leaves were steam-distilled and a light green oil was obtained with the following characteristics:—

Yield of the oil	.. 0·49 per cent
Specific gravity	.. 0·8058 at 15°C.
Refractive index	.. 1·478 at 20°C.

5. Iris kumaonensis Wall.—*Iris Kashmiriana* N.O. Iridaceae.—These two species of the plant grow wild in Kashmir and are also used medicinally. The dried roots of both the plants were steam-distilled but only traces of oil were obtained therefore, other properties could not be studied.

6. *Inula racemosa* Hook. N.O. Compositeae.—The plant is widely used by the Hakims and Vaids as an expectorant, diaphoretic and diuretic. The dried roots on steam-distillation yielded 0·38 per cent oil. The oil, on standing, solidified due to the alantolactone which forms a major portion of the oil.

Fumaria parwiflora Lamk.

This plant is known as *pitpapda* in Hindi and *Bansulpha* in Bengali. The plant occurs widely throughout the Indo-gangetic planes, growing in the rice fields during the cold weather and lower Himalayas up to 8,000 ft. It is very much used both in the Tibbi and Vaidic systems of medicine for varied purposes, such as, laxative, diuretic, alterative, tonic and febrifuge.

The whole plant was extracted with various solvents and the extractives on examination showed the presence of a fair amount of reducing sugars, potassium nitrate and alkaloids. Quantitative estimations gave 0·08 per cent

of total alkaloids. The alkaloid has been isolated as an amorphous powder. Further investigation of the alkaloid is being continued.

Pharmacological action.—One per cent solution of the alkaloid was used in pharmacological investigations. The alkaloid has no bacterio-cidal action as tested against B. typhus (T.E.D.). It kills the paramoecia in 18 minutes in a dilution of 1 in 20,000.

Action of the alkaloid on blood pressure and respiration was tested on rabbits and cats. 5 mgms. when administered intravenously hardly produce a marked fall in the pressure which however, soon regains its normal level. The fall of pressure is in no way effected even when the drug is administered after section of vagi or after the vagi are paralysed by atropine. Respiration however, becomes shallow and rapid for short period after administration of 5 mgms. intravenously. The drug seems to be promising and will be investigated further.

Lochnera pussila.

The plant belongs to Apocynaceae family and is called in Telugu 'Euripayalam'. The plant grows wild in South India and the Central Provinces. It has been reported to be toxic to animal life and cattle. The animals that eat it suffer from temporary partial blindness with urticarial rashes all over the body and are disabled for sometime. The disease is said to be not fatal and the animals recover in about two months.

Chemical analysis has been done. Examination of various extractives showed the presence of alkaloid in addition to resins and chlorophyll. Tests for the presence of hydrocyanic acid, saponins, volatile alkaloids and essential oils were negative. Quantitative estimation gave 0·22 per cent of the alkaloid.

Preliminary feeding experiments with the coarsely powdered drug did not reveal any poisonous effects.

OTHER RESEARCHES

35. Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

I. Study of regeneration of blood in donors, following donation of blood.

Effects of blood donation on the haemopoietic system of a group of 14 blood donors were studied with special reference to the regeneration of blood. All the donors were healthy young adults between the ages of 20 and 42 and all of them were perfectly fit for donation, both clinically and haematologically. There were only two female donors in this group. Of the 14 donors, 11 were Bengalees and 3 Anglo-Indians. The body weights of these donors varied from 44·1 Kg. to 82·7 Kg. with an average of 60·3 Kg. The amount of blood donated varied from 100 c.cm. to 400 c.cm. with an average of 218 c.cm.

Methods of investigation :—Complete cytological examinations of blood, including haematocrit reading, erythrocyte sedimentation rate and Van den bergh test was done in all the donors just before the donation. Post-donation examinations were carried out till the blood picture returned to the original pre-donation level. The blood picture of two of the donors were studied twice during two different donations, one in the course of 6 months and the other in the course of 11 months; the rest were studied only once during a particular donation. All the donors excepting one had donated blood more than once, number of donations varying from 2 to 17.

Results :—The maximum drop in haemoglobin was 1·79 gm. noticed 6 days after donation in a donor who in his 11th donation donated 400 c.cm. of blood. The maximum drop of 2,070,000 in R.B.C. was also seen in the same donor 3 days after the donation. This donor showed a definite microcytic anaemia with hyperbilirubinaemia; an occasional immature red cell (Normoblastic) was seen in the peripheral blood. The blood picture reached the pre-donation level in 40 days. The same donor in his 15th donation donated 225 c.cm. of blood with a drop of only 0·69 gm. in haemoglobin and 0·330,000 in R.B.C.; regeneration was complete within 4 days.

No drop in R.B.C. or haemoglobin was seen in a donor, who in his 2nd donation donated only 120 c.cm. of blood.

There was no reticulocytosis in any of the post-donation counts. No significant changes in the total and differential white cell count and erythrocyte sedimentation rate were seen in any of the donors. The indirect Van den bergh test was negative in all the cases, except in the one mentioned above.

The total recovery period for full regeneration of blood varied considerably, from 2 to 40 days, with an average of 9 days. Without any detailed statistical studies, it would appear that the bigger the donation, the greater is the fall in haemoglobin and R.B.C. irrespective of the number of donations.

II. Effect of organic arsenicals on the haemopoietic system of experimental animals.

Work on the effect of organic arsenicals on the haemopoietic system and liver of experimental monkeys was continued. As before the monkeys were given weekly intravenous injections of Novarsenobillon (May and Baker) in

the dosage of 0·03 G. per Kg. body weight. Complete blood picture including the haematocrit reading and Van den bergh test was studied, before the injections. Altogether 5 weekly injections of Novarsenobillon were given. Complete cytological examination of blood and Van den bergh test was done 24 hours after the injection and was repeated subsequently every other day. No significant anaemia, nor any evidence of liver damage, as shown by the indirect Van den bergh test (Quantitative), was noted in any of the monkeys.

III. Experimental production of granulocytopenia with amidopyrine.

Amidopyrine an analgesic and anti-pyretic drug on prolonged administration is said to induce granulocytopenia and even agranulocytosis. Experiments are now in progress to find out if the condition can be produced experimentally in animals. Four monkeys which did not show any wide variation in the total and differential white cell count were selected for the purpose. The animals were divided into two groups.

To one group of 2 monkeys a daily dose 0·04 gm. of the drug per Kg. body weight was given for 180 days. Another set of 2 monkeys was given a bigger dose, viz., 0·1 G. per Kg. body weight daily for 147 days.

In all the 4 animals of both these groups, slight neutropenia was produced during the middle of the period of observation; but the final counts do not suggest any leucopenia or neutropenia.

IV. Experiments to find out if induction of diarrhoea with cathertics would produce any change on the haemopoietic system.

These experiments have been started to see if chronic diarrhoea would produce microcytic anaemia, as a result of deficient absorption of the 'haematinic principle' from the hyper-irritable gut.

Two monkeys showing no gross variation in their weekly blood picture were selected for the purpose. Both of them were given extract cascara sagrada liquidum till diarrhoea was produced and then adequate dosage was continued to maintain the diarrhoea. One of the monkeys died on the 69th day of the experiment with slight reduction of haemoglobin and R.B.C. level, but without any evidence of macrocytosis. The other monkey is having diarrhoea for the last two months and a half and there has been a reduction of more than a pound in body weight, but as yet no significant anaemia has been produced.

36. Studies in tests of physical fitness in Indians under Dr. D. M. Telang at the Grant Medical College, Bombay.

The work was under suspension from January to July pending the appointment of a fresh assistant.

From the group of the three circulatory tests of physical fitness chosen studies on the Schneider Test and the Crampton Test were reported during the last two years. The third test—The McCloy Test—was studied this year.

The McCloy Test.

In this test the standing arterial diastolic blood pressure and the standing pulse rate of a subject are recorded. Using these two as the variable factors

a formula for the calculation of an index of physical fitness has been worked out by McCloy. The minimum index of fitness for American males is given as zero.

The test was done here on 418 male and 77 female subjects in the age group 16 to 29 years and comprising the following groups :—

Males—(1) 'no exercise' group of 249 of those who never took any regular physical exercise ;

(2) 'moderate exercise' group of 134 of those who were in the habit of taking some sort of daily exercise like walking or playing games ;

and (3) 'athletic' group of 35 of those who were indulging in regular athletic exercise in gymnasia.

Females—(1) 'no exercise' group of 58 ;

and (2) 'moderate exercise' group of 19.

There were none in the 'athletic' group amongst them.

The mean indices of fitness shown by these different groups are given in the following table.

Mean index of fitness—McCloy Test.

	'No exercise' group		'Moderate exercise' group		'Athletic' group	
	Males	Females	Males	Females	Males	Females
Mean index with p.e.	+0·77 ±0·45	−5·03 ±0·96	+4·4±0·6	−1·76 ±1·41	+8·21 ±1·19	..
Standard deviation	10·47	10·83	10·31	9·09	10·42	..
No. of subjects in group	249	58	134	19	35	..

A study of the influence of different factors on the index gave the following results :—

Influence of exercise—Amongst the male subjects the difference between the mean indices of the 'no exercise' and 'moderate exercise' groups is statistically significant while that between the indices of the 'moderate exercise' and the 'athletic' groups is very nearly so. This was obtained even after comparing the mean indices of persons grouped after keeping their diet, the time of day at which the test was done, and the diet as well as the time of day, constant.

One may therefore say that that the test grades the physical fitness fairly well assuming that regular exercise improves an individual's fitness.

Amongst the females the indices do not show quite a significant difference probably because there are few subjects in the 'moderate exercise' group.

Influence of sex—The corresponding male and female 'no exercise' and 'moderate exercise' groups show significant differences in their mean indices even after keeping the diet or the time of day for the test constant.

This probably suggests that sex markedly influences the test and that therefore the comparative standards for males and females need be different.

Influence of diet—Subgroups belonging to the same 'exercise' group but having different diets (vegetarian and mixed) could not be differentiated significantly by their means.

This suggests that diet has probably no influence on the test.

Influence of community—Subgroups, Deccanis, Gujarathis, Muslims, and Parsis belonging to the same 'exercise' group could not also be differentiated statistically from one another suggesting that the test is not affected by the community of the individual.

Influence of time of day—To determine this the test was repeated on a group of twenty-three subjects both in the morning as well as in the afternoon keeping the same time interval between the test and the previous meal.

No significant difference between their mean indices under the two conditions having been noted it may be said that the test is not influenced by the time of day at which the test is done.

From a comparison of the three circulatory tests of physical fitness so far reported on viz. the Schneider test, the Crampton test, and the McCloy test it may be said that the Schneider test is better than the other two in grading the fitness of an individual.

THE RESPIRATORY TESTS.

Studies on the four respiratory tests of physical fitness chosen viz., the expiratory force test, the breath-holding test, the persistence or fatigue or endurance test, and the vital capacity have now been undertaken.

37. Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras.

1. VACCINIA VIRUS.

The field experiments initiated in 1940 to ascertain the relative value of chick membrane lymph and routine calf lymph in the prevention of small-pox were continued.

491 primary and 2,216 revaccinations were done with chick lymph out of a total of 980 primary and 4,359 revaccinations done during the year in the experimental villages. The field results have not yet been obtained in a consolidated form for analysis.

The field results for the period 1st August 1943 to 31st December 1944 were analysed and a report forwarded to the Public Health Commissioner with the Government of India in March this year. 636 cases of primary vaccinations with membrane lymph verified gave a case success rate of 76·7%. The corresponding figures for calf lymph were 701 cases and 98·5% case success

rate. In revaccinations with membrane lymph a case success rate of 21·7% was obtained in 1936 revaccinations verified, while with calf lymph a case success rate of 36·5% in 1919 revaccinations verified was obtained.

In the experimental-villages there were 82 attacks of smallpox and 19 deaths. Of these, only 8 cases were among those vaccinated since October 1940, when the experiment was initiated. In the primary vaccination group there were 3 cases in the membrane lymph group. Of these, 2 were vaccinated during the incubation period and the result of vaccination in the third was not verified. In the revaccination group, 2 cases were in the membrane lumph group and 3 in the calf lymph group. The revaccination results of the 2 cases (membrane lymph) was recorded as 'failures' in the sense that there was no frank take; with calf lymph cases the results in 2 cases were not known, but in both, revaccination was done during the incubation period. The third calf lymph case was successfully revaccinated four years previously.

It would appear, therefore, that in spite of the presence of infection in the area and very high incidence of the disease in at least two of the experimental villages, the recorded attacks of smallpox in the population vaccinated since the commencement of the experiment were few and no difference was noted in the behaviour of the two types of lymph used.

2. Immunity to vaccinia following primary vaccination with chick membrane and calf lymphs.

350 children primarily vaccinated with membrane lymph and 373 children primarily vaccinated with calf lymph during the period July 1940 to February 1941 were available for study. The two groups of children were revaccinated with the respective lymphs, i.e., the membrane group children with membrane lymph, and calf lymph group of children with calf lymph. The results show that the immunity to infection with the homologous strain of vaccinia virus lasts for at least four years in 62·7% in 183 children vaccinated with membrane lymph and 74·8% in 267 children vaccinated with calf lymph.

Cross immunity experiments were not done. It was considered inadvisable to have in any village children vaccinated with both types of lymph. Such a procedure would have rendered difficult the interpretation of results obtained in the presence of an outbreak of smallpox, and the primary object of the experiment, viz., to ascertain the behaviour of the two types of lymph in the prevention of smallpox, would have been defeated.

A study of the case records of over a thousand cases of smallpox in Madras during 1944-45 was undertaken and a preliminary analysis has been made. The results will be communicated in due course.

3. Influenza.

Strains of Influenza Virus—A type PR8 strain and B type Lee strain—were brought from the Rockefeller Institute some time ago. Virus A strain has been established in the Institute and attempts have been made to manufacture of Influenza virus vaccine on a laboratory scale with the different methods recorded for the purpose. Virus B strain is under investigation. With the antigens from the two strains, attempts are now being made to diagnose the virus type prevalent here by examination of sera from cases of Influenza adopting the red cell agglutination technique.

4. TRAINING IN VIRUS WORK.

Three Science Graduates were appointed under the training scheme. They are receiving training in the general bacteriological technique and the special procedures employed in the study of filterable viruses.

38. Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

Since the inception of this enquiry 210 samples, obtained from various sources were submitted to bacteriological examination, for the isolation and identification of pathogenic anaerobes. This material included twenty-five definite cases of gas-gangrene. The incidence of organisms responsible for these cases was as follows :—

Cl. welchii	10 cases
Cl. welchii along with Cl. septique ..	3 cases
Cl. septique alone	6 cases
Cl. histolyticum alone	4 cases
No organisms	2 cases
Total ..	25 cases

It was evident that Cl. welchii alone or in combination with other anaerobes was the predominant causative organism of gas gangrene, a finding which is in accordance with the common belief. Being the most important agent of this disease it was decided to study these strains of Cl. welchii in greater detail. Out of the 13 cases of gas gangrene accounted by Cl. welchii infection 8 expired, 4 recovered and the remaining one was discharged against advice. All these 13 strains and 6 more strains of Cl. welchii (isolated from samples of guineapig and human stools) were capable of killing the mice when varying doses of fresh cultures were injected intramuscularly. Studies on filtrates, obtained from their growth in suitable media, showed, that filtrates of all, excepting one strain, failed to exhibit any lethal action on white mice. In order to find out the cause of this incongruity in the results it became necessary to study the other factors which take part in production of the infection. With this aim in view the lecithinase activity of these strains was studied. Their lecithinase activity was found to vary, but the general impression given by this study was that one of them produced powerful lecithinase. Their haemolytic activity was next investigated and it was found that every one of them was markedly haemolytic. When however their capacity to produce hyaluronidase was tested, it was noticed that with the exception of three strains every one of them showed a pronounced reaction.

The results obtained so far suggest that there exists some discrepancy in pathogenicity and toxin production of Cl. welchii. For example it was found that strains of Cl. welchii isolated even from fatal cases human gas gangrene were very poor lecithinase producers.

Pathogenesis and the lethal outcome of Cl. welchii infection thus affords a complex problem which even to this day remains unsolved. It is likely that the lethal effect of Cl. welchii may be due to either (1) their invasive capacity

(2) or their capacity to produce powerful factor i.e. lecithinase which exhibits lethal haemolytic action (3) or due to lethal factor derived from muscle-tissue destroyed and not to any known components of toxic factors. Recently there is a growing tendency to ascribe the general clinical manifestations of Cl. welchii infection to the myo-necrosis produced. It has been suggested that Cl. welchii during their growth produce a substance known as 'Collagenase' responsible for the myo-necrosis which is a predominant feature of infection by this organism. Further experimental work is needed to confirm this hypothesis and to exclude the role of invasive capacity and α factor produced by Cl. welchii. Hence the work of these strains of Cl. welchii. isolated during the course of this enquiry is being carried out further. Experiments have been planned and undertaken—

(1) to study the behaviour of these organisms in experimentally produced gas-gangrene infection, with especial attention to the development of septicimic stage.

(2) to note the effect of animal passage and prolonged cultivation in presence of lecithin on the lost virulence and lecithinase production of some of these strains of Cl. welchii.

(3) to determine the collagenase activity of filtrates of Cl. welchii in addition to their lecithinase and haemolytic activity in definite units.

This work, which is in the experimental stage, is in progress and the results obtained will be communicated later on.

39. Enquiry on the action of snake venoms and other constituents on the biochemical activity of tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

1. Effect of cobra venom on the aerobic oxidation of sodium pyruvate.

It has been reported previously that cobra venom inhibits the aerobic oxidation of glucose, sodium lactate and sodium succinate by brain cells of pigeons. In continuation of this work, the effect of snake venom on the aerobic oxidation of sodium pyruvate was investigated. The sodium pyruvate was prepared by the method of Stedman (Biochem. J. 1938, 32, 2031). The experimental procedure was the same as described in the previous report. Minced pigeon's brain (about 100 mg.) suspended in 2·0 c.c. of phosphate-saline buffer pH 7·4 was placed in the conical flasks of the Warburg's manometric apparatus and 1·0 c.c. of 0·1 M. sodium pyruvate in phosphate-saline buffer containing the desired quantity of cobra venom was placed in the side bulb. The entire system was filled with oxygen and placed in a thermostat at 37·3° C. thus allowing the system to respire freely for 2 hours, when the substrates originally present in the brain cells were depleted considerably. The substrate from the side bulb was then tipped into the flask and the rate of absorption of oxygen was determined at intervals of 15 minutes. The requisite number of controls were also set up side by side. A marked inhibiting effect of cobra venom on the aerobic oxidation of pyruvate was observed. With 0·1 mg. of venom, the inhibition was about 40-45 p.c. in 1 hour, while with 0·5 mg. and 1·0 mg. of venom, the inhibition was about 80-90 p.c. in 1 hour. It is thus evident that after a certain

dose of venom, the inhibition does not increase any further i.e. a point of saturation is attained.

It is interesting to know that in certain respects, cobra venom resembles the narcotics, while it differs from them in others. The narcotics are known to inhibit the aerobic oxidation of glucose, lactic acid, and pyruvic acid, but have no appreciable effect on the oxidation of succinic acid. Cobra venom on the other hand, inhibits the aerobic oxidation not only of glucose, lactic acid and pyruvic acid, but also of succinic acid by brain cells.

It is also intended to ascertain as to how far the inhibition of oxidation of the substrates mentioned above by the brain cells is reversible, i.e. how far the brain cells regain their original activity if the venom is removed by washing. It may be mentioned here that the action of narcotics on the brain cells is reversible.

2. Effect of cobra venom on cytochrome oxidase.

Since the cytochrome-cytochrome oxidase system plays a very important part in biological oxidation, the effect of cobra venom on this system was also investigated. Pigeon's brain tissue was used as the source of the enzyme and the substrate was 1% p-phenylene diamine hydrochloride solution neutralized to pH 7·0 just before use. Since a pigeon's brain contains various enzymes and also various substrates like glucose, lactate etc. the disturbing effect due to these enzymes and substrates was sought to be eliminated by shaking the minced brain with phosphate-saline buffer, pH 7·4 for 2 hours at 37·5° C. in an atmosphere of air. By this procedure, the residual respiration was found to be reduced considerably and also sufficient time was allowed to the venom to exert its effect on the cell. The substrate i.e. 1% p-phenylene dyamine hydrochloride adjusted at pH 7·0 was then added in such amount that the final concentration was 0·33%. Controls were maintained showing the residual respiration of the normal brain and also that of the brain in the presence of venom.

Various doses of venom starting from 0·1 mg. were used and it was found that even a dose of 0·1 mg. produced an inhibition of about 70 p.c. Addition of increasing doses of venom did not appreciably increase the inhibition any further.

It is intended to investigate the effect of cobra venom on the oxidation of substrates like xanthine, d-glutamic acid, malic acid and fumaric acid by the brain cells of pigeons. This may enable us to explain the mechanism of the toxic action of cobra venom.

3. Study of the physico-chemical properties of cardiotoxin.

It has been reported previously that cobra venom contains an active principle which can bring about the stoppage of the movement of the heart. This active principle has been termed cardiotoxin. In the report submitted last year, a method of isolating the cardiotoxin from the crude venom has been described. This year, the iso-electric behaviour and approximate molecular weight of the pure cardiotoxin fraction have been investigated. The nature of the electrical charge and migration of the particles of cardiotoxin at different pH have been investigated by the micro-cataphoretic as well as by the moving boundary method. The iso-electric point of cardiotoxin has in this way been found to be in the neighbourhood of pH 8·2.

The approximate molecular weight of the cardiotoxin has been estimated by studying its rate of diffusion at different pH at a constant temperature. The approximate value of the molecular weight has been found to be 33,000.

It is intended to undertake a comparative study of the effect of cardiotoxin with that produced by saponin, saponin like substances and drugs of the digitalis group on the isolated frog's heart. Whether the effect produced by cardiotoxin on heart muscle is reversible or not will also be investigated.

40. Enquiry on typhus under Dr. S. R. Savoor at the Haffkine Institute, Bombay.

I. Work on typhus in Bombay.

(a) From the 1st of January till the 30th of November, 1945, Weil-Felix reactions were carried out on 1497 samples of sera. Of these, 115 gave positive agglutination reactions against Proteus OX19 in dilutions of over 1/250.

Till the end of May the Weil-Felix reactions were carried out using only the (alcoholised) suspensions of Proteus OX19. Since then suspensions of Proteus OX2 and OXK have been used, with the result that two cases of typhus, OX2 in type, and nine OXK in type, were spotted. It is of interest, therefore, to note that in Bombay and its suburbs all the three types of typhus infections corresponding to the three types of Proteus X (Felix's classification) are met with. Of the nine cases, five were in individuals who had never been outside the city. Two of the five Bombay cases showed the typical tsutsugamushi escar.

Hitherto, the cases of scrub typhus recorded in literature had exclusively been amongst residents in the country side. The occurrence of scrub typhus in Bombay, therefore, indicates that the vector and the ecological conditions favourable to its development exist even in a metropolitan area.

(b) *Isolation of fresh strains* :—

(i) Attempts were made to isolate strains of typhus, OX19 in type, from five cases. Two succeeded. The strains were of the 'orchitic' type producing tunica lesions in male guinea-pigs and corresponded with the two strains isolated in 1943 and 1944.

(ii) Attempts have been made to isolate strains of typhus from two cases giving Weil-Felix reactions with Proteus OX2. No strains were isolated.

(iii) Mice were inoculated with blood from four cases of scrub typhus. Three strains were isolated.

(c) Epidemiology :—It has been shown in previous years that (i) a strain of typhus had been isolated from the brains of rats trapped in a house where a case of typhus had recently occurred, and (2) *X. cheopis* transmitted two strains of human origin from mouse to mouse under laboratory conditions. These findings indicate that rat and rat-flea (*X. cheopis*) are concerned in the transmission of typhus in Bombay. However, it appeared desirable to isolate strains of typhus, by the normal means of transmission, from the fleas on Bombay rats.

Six experiments have so far been performed. None of the experiments yielded a strain.

(d) An analysis of the symptoms of typhus in Bombay is being studied.

II. Work on scrub typhus (XK typhus).

(i) Chemotherapy of experimental scrub typhus in mice: Batches of mice intra-peritoneally infected with ten lethal doses of virus were fed with 5 representative compounds which are heterocyclic derivates related to sulphathiazole and sulphadiazine and derivates of diphenyl sulphones. These drugs synthesised at the Haffkine Institute have been shown to possess chemotherapeutic action against experimental pneumococcal and streptococcal infections in the mouse. None of the drugs had any chemotherapeutic action against (XK) typhus in the mouse.

(ii) Cultivation of R. tsutsugamushi in vitro:—Attempts were made to see whether *R. tsutsugamushi* could be grown on a medium containing cystine at different temperatures under acrobic and anacrobic conditions.

A diplo-bacillary organism was grown at 27°C. In the earlier generations this organism could not be grown on the usual media, including media containing blood or serum. Investigations showed the organism to be non-pathogenic to the mouse and conferred no immunity against experimental scrub typhus infection in the mouse.

(iii) Experimental rickettsial pneumonia in the rabbit:—Just about a year ago a vaccine was prepared at the National Institute at Hampstead out of the experimentally produced (rickettsial) pneumonic lungs of cotton rats. As cotton rats are not available in India, attempts were made to see whether a rickettsial pneumonia could be produced in the rabbit. The virus was inoculated by the intra-tracheal route in volumes of 5-8 c.c. in normal rabbits or in smaller amounts in rabbits in which a temporary pulmonary oedema had been produced by various means, and in rabbits whose lungs had been damaged by bromine vapour. The source of the virus was the peritoneal exudate of infected mice. (M.L.D. of mouse = 0·00001 c.c. of peritoneal fluid) or virus grown in the yolk sac of the developing duck. To prevent septic infection, the rabbits were given twice daily sulphathiazole per mouth. Twelve experiments were done. Apart from a fleeting consolidation that resulted following intra-tracheal inoculation, no morbid changes resulted. The rabbits did not die of infection.

(iv) Experiments in the mouse have shown that between the 'Naigaum" strain of scrub typhus, isolated in Bombay, and the "Calcutta" and the "Ceylon" strains there is a complete and reciprocal cross-immunity.

41. Enquiry into vaccine lymph under the Director of Public Health, Bombay, Poona.

During the year under review studies on the serological and immunological aspects of immunity after vaccination, were conducted.

Serological studies.

The level of immunity after vaccination is indicated by the nature of response to a subsequent revaccination. *Force et al* have recorded distinct inter-relationship between the response of an individual to vaccination and the presence of virus neutralizing bodies in his blood. Attempts were made in this investigation to ascertain if a similar relationship existed between the response to revaccination and the agglutinin or precipitin content of the serum.

Method & materilas.

Sera were first collected from the blood of some members of the inferior staff of this Institute. They were then vaccinated and their response noted. The various sera were first tested for flocculation against an antigen prepared from lapine collected 72 hours after vaccinating rabbits. The antigen was prepared by centrifuging in an angle centrifuge a saline extract of lapine and then suitably diluting the clear supernatant.

Several tests for flocculation with different dilutions of the antigen and also the sera were entirely negative.

Agglutination tests were then carried out. For the antigen a suspension in buffer solution of elementary bodies obtained from vaccine pulp of calves by differential centrifugalization was used. After preliminary tests the sera were tested in dilutions of 5, 10, 20 and 40 in buffer saline. The mixtures in agglutination tubes were incubated in a water bath at 52°C. for 18 hours, then kept at room temperature for 24 hours and the results then read.

The response to revaccination of the various individuals and the agglutination titres of their respective sera are tabulated below :—

Table showing the agglutination and revaccination results.

Serial No.	Agglutination titres of sera				Response to revaccination
	5	10	20	40	
3	+	—	—	—	Immune reaction
4	+	—	—	—	—do.—
5	++	++	+	—	Vaccinoid
8	++	+	—	—	Immune reaction
10	++	+	—	—	—do.—
11	±	—	—	—	—do.—
12	+±	+±	+	—	—do.—
13	++	+	±	—	—do.—
16	+±	±	—	—	—do.—
17	+±	+	—	—	—do.—
19	++	+	—	—	—do.—
21	++	+	±	—	—do.—
22	±	—	—	—	—do.—
24	+±	+	±	—	Vaccinia
25	++	+	±	±	Immune reaction
26	+++	+++	++	+	Vaccinoid
27	++	+	+	—	—do.—
28	++	++	++	+	Immune reaction
31	++	+	—	—	—do.—
32	++	+	±	—	—do.—
33	++	++	+	—	Vaccinia
34	±	—	—	—	Immune reaction
35	+	+	—	—	—do.—
36	++	++	+	+	Vaccinoid
37	+±	+	—	—	Negative
38	—	—	—	—	Immune reaction

It will be seen from the above that there is absolutely no correlation between the immune status of the individual as assessed by the nature of his response to revaccination and the agglutination and flocculation titres of his serum, while according to *Force et al* there is a parallelism between the virus neutralizing antibody content in the blood and the response to vaccination. This is explained by the work of *Parker and Rivers* who found that the production of agglutinins and precipitins did not run parallel with that of neutralizing antibodies and that the antibody content gave no indication of resistance to reinfection.

Immunological studies.

A fairly large amount of data was collected during the year, on the response of individuals to revaccination, with varying intervals, after the previous primary vaccination or revaccination.

In the earlier stages of this investigation all persons, whatever the interval, were included in the study. The individuals were classed into 4 groups based on the interval period, viz., " 5 years & less ", " 6 to 10 years ", " 11 to 20 years ", and " over 20 years ". However the vaccination histories of the last two groups could not be relied upon and in addition these 2 groups constituted a relatively small percentage of the total number of persons revaccinated.

The appraisal of the results confined to the first two groups furnished a fair indication of the duration of immunity after successful primary or revaccination. It was observed that while in the " 5 years and less " group the ratio of individuals with absolute protection to those showing partial and no protection, was 2·25 : 1, that in the " 6-10 years " group was 1 : 3·2. The relevant figures are tabulated below :—

Interval between the present revaccination & the last vaccination or revaccination	No. showing negative results	No. showing immune or immediate reaction	No. showing vaccinoid or accelerated reaction	No. showing vaccinia or primary reaction
(1) 5 years & less	5 (1·7%)	197 (68·1%)	80 (27·6%)	7 (2·3%)
(2) 6 to 10 years	3 (1·1%)	62 (23·3%)	111 (41·7%)	90 (33·9%)

In view of the above results it was decided to confine subsequent studies to a detailed investigation of the " 5 years and less " group and also to ascertain if the immunity curves after primary vaccination and revaccination follow identical trends.

All the individuals were revaccinated with 2 insertions on one forearm, with a control mark on the other, and the results were classified as usual. No children at all were available for revaccination with an interval of 1 year after primary vaccination and a few with a 2 year interval, obviously due to reluctance on the part of the parents to submit their recently vaccinated young children for revaccination. However an appreciably larger number was available with intervening periods of 3 years and more and better still was the cooperation from the older children who had already been revaccinated prior to this investigation. The results are tabulated below :—

Results of first revaccination after varying intervals after primary vaccination.

Interval between primary vaccination & the present first revaccination	No. showing immune or immediate reaction	No. showing vaccinoid or accelerated reaction	No. showing vaccinia or primary reaction
1 year	None available	None available	None available
2 years	8 (80%)	2 (20%)	0
3 years	21 (47·7%)	20 (45·4%)	3 (6·9%)
4 years	16 (34·8%)	22 (47·8%)	8 (17·4%)
5 years	11 (29·7%)	17 (45·9%)	9 (24·4%)

Results of revaccination after varying intervals after the last revaccination.

Interval between the last revaccination and the present revaccination	No. showing immune reaction or immediate reaction	No. showing vaccinoid or accelerated reaction	No. showing vaccinia or primary reaction
1 year	76 (90·5%)	8 (9·5%)	0
2 years	30 (75%)	10 (25%)	0
3 years	71 (64·5%)	37 (33·6%)	2 (1·9%)
4 years	11 (40·8%)	15 (55·5%)	1 (3·7%)
5 years	2 (28·5%)	4 (57·2%)	1 (14·3%)

The above results afford an appreciable indication of the immunity trends after successful primary and revaccination. It will be noticed that absolute immunity as indicated by the ' Immune ' or ' Immediate reaction ' falls off more gradually with lengthening intervals after revaccination than after primary vaccination. Similarly a steeper rise with lengthening intervals, in the percentage of non-immunes—as indicated by the ' vaccinia ' response—is observed after primary vaccination than after revaccination. This statement will be clearly appreciated by the following tabular arrangement of the relevent percentages :—

Immunity trends after Primary and Revaccination.

Interval between two consecutive vaccinations	Percentage of individuals showing immune or immediate reaction = absolute protection		Percentage of vaccinated individuals showing vaccinoid or accelerated reaction = partial protection		Percentage of individuals showing vaccinia reaction = No protection	
	Primary vaccination group	Revaccn. group	Primary vaccn. group	Revaccn. group	Primary vaccn. group	Revaccn. group
1	2	3	4	5	6	7
1 year	None available	90·5%	None available	9·5%	None available	0%
2 years	80%	75%	20%	25%	0%	0%
3 years	47·7%	64·5%	45·4%	33·6%	6·9%	1·9%
4 years	34·8%	40·8%	47·8%	55·5%	17·4%	3·7%
5 years	29·7%	28·5%	45·9%	57·2%	24·4%	14·3%

It would be interesting to evaluate these results statistically to determine a 'figure' for the duration of "adequate immunity" both after primary vaccination and revaccination and see how far such a figure conforms to the current ideas regarding the duration of immunity. Obviously more data would be helpful and efforts will be made to present the same in a subsequent report.

The study of the response to revaccination after a previous revaccination cannot be quite complete without a knowledge of the nature of the reaction to the previous revaccination. It would be interesting to ascertain to what extent the response to the present revaccination is influenced by the nature of the reaction to the previous revaccination. In other words does the type of the reaction to revaccination influence the duration of immunity and if so, in what way? Records pertaining to revaccination results of a large majority of the individuals now under study and who were revaccinated prior to the commencement of this investigation are not available. Hence an analysis of the results on these lines has not been possible in this report. It is proposed to pursue investigation of this aspect of the question in subsequent studies.

42. Enquiry into the fraction of liver most suitable for the treatment of tropical macrocytic anaemia under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

Though additional grant for the purchase of a large vaccum distillation apparatus was sanctioned early this year the apparatus could not be put into working order till recently. Consequently additional staff budgetted for the current year, was appointed only recently and therefore full advantage of the additional grant could not be taken of this year.

The nutritive value of the liver extracts will depend to a great extent on the proportion of moisture, protein, fat, carbohydrate and mineral matter in which they are present in the livers of different animals. Investigations were therefore undertaken to find out the proportion of these factors in the livers of cow, sheep, buffalo and pig. It was seen that the buffalo liver was most rich in total protein, carbohydrate and mineral matter, the sheep liver was most rich in fat and iron contents while the moisture content was the highest in the pig liver.

Proteolysed liver.

In storing proteolysed liver in the cool chamber in sealed tubes over a month, a precipitate used to be found in the tubes which gave a cloudy appearance. This was found to be due to denaturing of metaproteins in the proteolysed liver, which ultimately appeared as precipitate. To avoid this, removal of metaproteins from the proteolysed liver concentrate has now been adopted by adjustment of the hydrogen ion concentration of the digested extract at pH 5 and by subsequent heating at 70° C. for about 10 minutes. By this treatment considerable amount of metaprotein is precipitated out and the clear filtrate is then treated under usual procedure. Clinical trials with proteolysed liver have been continued on suitable cases of tropical macrocytic anaemia in the Carmichael Hospital for Tropical Diseases. Thirty-one cases of tropical macrocytic anaemia have been treated so far. Last year proteolysed liver extract derived from 50 grms of raw liver was given daily; it is now found that

proteolysed liver extract derived from 100 grms. of raw liver is well tolerated. The present day procedure is to start with proteolysed liver extract derived from 50 gms. of raw liver and to increase the doze gradually till extract derived from 100 gms. of raw liver is given daily. This is continued till the red cell count reaches the 2·5 million level. On analysis of the 31 cases treated with proteolysed liver extract, it was found that the response to treatment with proteolysed liver in uncomplicated cases of tropical macrocytic anaemia was generally of the same order as that of potent injectible crude liver extracts. Cases with megaloblastic reaction of the marrow respond better with proteolysed liver than the cases with normoblastic reaction of the marrow.

A paper entitled 'Proteolysed liver extract in the treatment of tropical macrocytic anaemia' is almost ready for publication.

FRACTIONS OF LIVER EXTRACT.

Investigations about the efficacy of the different fractions W, G, and V in a small number of cases definitely show that the fraction W is superior to fraction G in the treatment of tropical macrocytic anaemia, while fraction V did not seem to have any effect at all.

43. Enquiry on serum standardisation under the Director, Central Research Institute, Kasauli.

The work of this enquiry has been directed chiefly to working out a more satisfactory method for the assay of antivenom serum than any at present available. The current method in use by the Central Research Institute, Kasauli, depends on the saving value of antivenom serum in pigeons injected with mixtures of venom and serum. This method has the disadvantage of being somewhat inaccurate and erratic, and there is a very real need for a more precise method of assay. Attempts have been made to obtain a more reliable method by using other animals and by investigating the effects of using different routes of injection of the venom-serum mixtures. Experiments with the common sparrow proved unsatisfactory for standardization purposes for various reasons, among which was the high mortality of sparrows kept in captivity. Similar experiments were carried out in the Indian red munia (*Sporaeginthus amandava*) and, although these birds were found to do well in captivity and to be susceptible to the action of venoms, they showed considerable variation in their resistance to the effects of venoms. Guinea-pigs and mice were found to give more uniform results and up to the present, the most constant results have been obtained in guinea-pigs weighing between 240 and 320 gms., the serum-venom mixture being given by the intracardial route.

2. EXPERIMENTS ON THE ASSAY OF ANTIVENOM SERUM FOR POTENCY AGAINST COBRA VENOM.

Preliminary experiments were undertaken to determine suitable methods for the isolation of the two main fractions in cobra venom, namely neurotoxin and haemolysin. The neurotoxin fraction was obtained by eliminating the haemolysin fraction by :—

(a) precipitation of venom solution by sodium-chloride or ammonium sulphate;

(b) precipitation of haemolysin by the addition of trichlor-acetic acid at 0°C. to 1°C.;

(c) absorption of haemolysin on Seitz-pads or charcoal; and

(d) destruction of haemolysin by the addition of sodium-hydroxide

The most suitable method for the isolation of cobra neurotoxin was by the addition of sodium-hydroxide to cobra venom solution. This was most conveniently done at room temperature in two stages, the excess of alkali being neutralized by dilute acetic acid. The best method for the isolation of the cobra haemolysin was by 20% saturation with sodium-chloride. The titre of samples of antivenom serum collected from 19 different horses was determined by *in vivo* methods in parallel with the antihaemolysin titre as determined by *in vitro* methods. In 15 of these sera, the antihaemolysin content did not correspond closely with the protective action of antivenom serum as determined by *in vivo* titration. In the remaining four sera, the antihaemolysin content was found to be very low, while their protective action *in vivo* showed a wide range of variation. The action of both neurotoxin and haemolysin fractions of cobra venom in the presence of antivenom serum was investigated by the following *in vitro* methods:—

(1) *Action on Paramaecium caudatum*:—Preliminary experiments showed that haemolysin was lethal to paramaecium in high dilutions, whereas neurotoxin was lethal only in comparatively low dilutions. Suitable mixtures of cobra neurotoxin and antivenom serum were added to cultures of paramaecium, but the lethal action of cobra neurotoxin, determined in this way, was not found to correspond closely with the titre of the antivenom serum, as determined by *in vivo* titration, when the neurotoxin-serum mixture had been kept for one hour at room temperature. More regular results were, however, obtained when the mixture had been incubated at 37°C. for 18-24 hours.

(ii) *Flocculation test*:—It was possible to demonstrate flocculation due to the interaction of the haemolysin fraction with antivenom serum, but no flocculation occurred with the neurotoxin fraction in the presence of antivenom serum.

(iii) *Modified Nagler's reaction*:—The addition of egg-yolk to a solution of the haemolysin of cobra venom produced turbidity or even definite flocculation after incubation at 37°C. overnight, due to the interaction of haemolysin with lecitho-vitellin. No such turbidity or flocculation was observed when this experiment was repeated with a solution of the neurotoxin fraction.

(iv) *Complement fixation reaction*:—Fixation of complement in a system composed of cobra neurotoxin *plus* antivenom serum *plus* complement mixture was observed to occur, but the results obtained could not be correlated with the titre of the antivenom serum used as determined by *in vivo* tests. Trials were made with mixtures maintained at 37°C. for 1½ hours, as well as with mixtures maintained at refrigerator temperature overnight.

3. Experiments on the assay of antivenom serum for potency against daboia venom.

Attempts were made to isolate the toxic fraction or fractions of daboia venom with the ultimate object of using these fractions in devising an *in vitro* test. The following methods were used for this purpose: (a) precipitation with

trichlor-acetic acid at low temperature, (b) addition of pepsin + dilute hydrochloric acid for the differential destruction of haemolysin, (c) destruction of haemolysin by contact of venom solution with dilute alkali. No difficulty was experienced in isolating the fraction or fractions containing haemolysin, but no evidence was obtained of a non-haemolytic toxic fraction, in fact no worker has so far succeeded in splitting the haemolysin containing constituent.

Different samples of antivenom serum were titrated in guinea-pigs injected by the intra-cardial route with suitable mixtures of daboia venom and antivenom serum and the same sera were tested by the following *in vitro* methods :—

(i) *Action on Paramaecium caudatum* :—Preliminary experiments showed that only high concentrations of daboia venom were toxic to paramaecium. The addition of egg-yolk to the venom + paramaecium mixture markedly enhanced the action of the venom, but the neutralization experiments carried out with venom + antivenin mixtures gave far too irregular results to be of value.

(ii) *Complement—fixation reaction* :—By employing relatively large amounts of venom and antivenin, the presence of complement fixing antibodies was demonstrable in low concentrations. This test was not considered to be of practical value.

(iii) *Coagulation reaction* :—The presence of a coagulant in high concentration in daboia venom is well-known. It has been suggested that the coagulant is responsible for the lethal effect of the venom. In a preliminary experiment the action of a sample of daboia venom on citrated sheep blood was studied after the addition of calcium chloride. Then mixtures were made containing suitable dilutions of venom and antiserum, citrated sheep blood and an appropriate amount of calcium chloride solution were added and the mixtures were examined at intervals for coagulation. The results were not always uniform, the mixture containing a relatively low dilution of antivenin coagulated whereas other tubes containing higher dilutions of serum did not show any coagulation. The results were far too irregular to be of practical utility.

(iv) *Flocculation reaction* :—The flocculation reaction could be demonstrated by the addition of varying amounts of antisera to appropriate amounts of venom and by keeping the mixtures at 45°C. in a water-bath. The flocculation time varied from 6 minutes to 149 minutes. The presence of two zones was observed in 3 out of 20 sera. On taking the first zone only into consideration, some correlation was observed between the *in vivo* figure and the titre determined by flocculation in 8 out of 18 sera.

(v) *Anti-haemolysin titration*:—In a preliminary experiment the action of daboia venom on a suspension of goat-cells+egg-yolk was studied. Suitable dilutions of venom and antivenin were mixed and goat-cell suspension+egg-yolk were added. After keeping the mixtures at 37°C. for one hour and then at room temperature for two hours, the results were noted. On comparison with the *in vivo* figure, it was found that there was a fair degree of correlation between the *in vitro* and *in vivo* figures in 13 out of 20 sera.

(vi) *Modified Nagler's reaction*:—Mixtures of appropriate dilutions of venom and different samples of antisera were prepared and a pre-determined amount of filtered egg-yolk was added. The results were read after incubating the mixtures overnight at 37°C. Out of 21 sera examined 15 gave a figure which corresponded fairly closely to the neutralizing titre in guinea-pigs.

The flocculation reaction was found to be inferior to the anti-haemolysin titration and the modified Nagler's reaction. The titre determined by the modified Nagler's reaction was on the whole comparable to the anti-haemolysin titre, but the former reaction appeared to be a better guide to the *in vivo* figure.

The work on this enquiry was carried out by Dr. R. K. Goyal, MB., D.Sc., Ph.D., M.R.C.P., M.R.C.S., F.R.S.E., Assistant Director, Central Research Institute, Kasauli.

44. Enquiry on Kala-azar under Dr. P. C. Sen Gupta at the School of Tropical Medicine, Calcutta.

1. Investigations on the complement-fixation for kala-azar.

(a) About the beginning of the year, a modified technique of complement fixation test for kala-azar using an antigen prepared from Kedrowsky's acid fast bacillus according to the method described by Witebsky, Klingenstein & Kuhn was developed by the officer-in-charge of the enquiry. It has been tested out in a large series of cases and found to be satisfactory. A paper containing the full details of this modified technique has been published in the Indian Medical Gazette, August, 1944.

(b) The details of the method of preparation of the antigen from the different acidfast bacilli, for the complement fixation test for kala-azar have been further worked out. A paper on this subject in conjunction with Dr. Dharmendra and Mr. R. Bose of the Leprosy Research Department, School of Tropical Medicine, is under preparation.

2. Chemotherapeutic investigations on the aromatic diamidines used in the treatment of kala-azar.

(a) The clinical trials of a new aromatic diamidine—4 : 4′ diamidinodiphenyl ether, (phenamidine, M. & B. 736) have been completed. It has been found that this drug has a considerable degree of activity in the treatment of Indian Kala-azar, the immediate cure rate being about 86% and the permanent cure rate about 64%. These figures are, however, distinctly lower than what were obtained with the best of the pentavalent antimonials or with 4 : 4′ diamidino-stilbene. A paper on the subject is under publication in the Indian Medical Gazette.

(b) The work on certain toxic effects of diamidino stilbene commenced three years ago has been taken up again. Attempts are being made to produce diamidino stilbene neuropathy in rhesus monkeys who have been given a series of injections of the drug in 2 mgm. per kilo body weight doses. About 3 to 4 months after the completion of the injections—the monkeys will be destroyed and the neurological changes in the nervous system studied.

3. Chemotherapeutic investigations on sodium antimony^{v} gluconate in the treatment of Indian kala-azar.

50 cases of kala-azar (and two post kala-azar dermal leishmaniasis) were treated during the year with sodium antimony^{v} gluconate [stibatin (glaxo), containing 20 mgm. of Sb per c.c. of the solution]. The immediate results have

been very satisfactory. So far three cases have relapsed but the period of observation of most of the cases has been less than 6 months and more relapses are possible. A paper containing the *preliminary* observations on the treatment of kala-azar with sodium antimony gluconate is under publication in the Indian Medical Gazette.

4. THE TREATMENT OF CANCRUM ORIS COMPLICATING KALA-AZAR.

The use of penicillin, intramuscularly and locally, in the treatment of cancrum oris complicating kala-azar was commenced about the beginning of the year. The results have been very satisfactory in all the cases treated. A paper on this subject is under publication in the Indian Medical Gazette.

45. Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

Gastrodiscoides hominis, an intestinal fluke of man and pigs is fairly common in some parts of Assam and the North-eastern frontier of India. This worm causes ill health in a large number of persons over a wide area and has caused death in untreated people, specially in children. The object of this inquiry was to find out the life-history of the fluke to enable adoption of suitable preventive measures.

The adult flukes and their eggs were collected from the intestinal contents of infected pigs received from the local slaughter house. The eggs were concentrated by repeated sedimentation of the faeces in water and the flukes were dissected to get a further supply of eggs. In water, the eggs hatched from the fifteenth day onward upto three months. No difference in the rate of hatching was observed in either tank water, tap water or distilled water.

The snails were left in contact with the freshly hatched miracidia for 24 hours and observed for the possible development of the miracidia into cercariae. No actual penetration of the snail by the miracidia was seen.

The following one hundred and forty-three snails were thus exposed, the number in brackets indicating the number of snails used :—*Bithynia pulchella* (8), *Gyraulus convexiusculus* (20), *Indoplanorbis exustus* (7), *Lymnoea luteola* (4), *Neritina sp.* (30), *Melanoides lineatus* (39), *Melanoides tuberculatus* (9), *Vivipara bengalensis* (26). Of these, thirty-seven died within three weeks; sixty-eight died between three to thirteen weeks. Thirty-eight snails are still alive and are under observation. No cercariae were seen emerging from any snail nor any sporocysts or rediae found on dissection of those that died during the period of observation. These snails therefore do not appear to be the possible intermediate hosts and a search for other snails is indicated.

INDIGENOUS ANTHELMINTIC DRUGS.

The drugs which have been tested so far are the seeds of *Butea frondosa*, fruit of *Embelia ribes* and *Kamala* powder.

Hookworm infestation.—Powdered embelia, 60-130 grains given in a single dose cured 1 out of 11 cases. Combined Butea and Embelia, 60-150 grains of each cured 5 out of 113 cases. The value of these two drugs is negligible against hookworms.

Ascaris infestation.—With embelia, 30-180 grains given in a single dose, 4 cases out of 20 were cured. With combined Butea and Embelia (60-150 grains of each), 36 cases out of 73 were cured by a single dose. With Butea alone (10-60 grains), 11 out of 37 were cured by one treatment. The anthelmintic effect of combined Butea and Embelia in ascariasis thus compares very favourably with that of santonin or oil of chenopodium which are much more costly than these common indigenous drugs.

Taenia (tapeworm) infestation.—Twenty-three cases were given embelia alone in 120 grains dose. Three were cured, six were not and the after-history of the rest was not available. Embelia and Kamala, 120 grains of each, in a single dose was administered to eighteen cases. Nine were not cured, the rest did not report themselves. With combined Butea and Embelia, 120 grains of each given to six cases, 4 were not cured, and the remaining 2 did not come for further treatment. These drugs do not appear to have any specific action on tapeworms. Fourteen cases of *Hymenolepis nana* infestation were treated with these drugs singly and in different combinations without any effect on the worms.

46. Enquiry into methods for the manufacture of penicillin and other problems connected with it under the Director, Haffkine Institute, Bombay.

Six strains of Penicillium notatum were tested in surface cultures for their capacity to produce penicillin by growing them in the modified Crapek-Dox medium. The pH of the culture fluid, the penicillin titre and the dry weight of the mould were followed daily. The assays of penicillin potency were done by the Oxford plate method and occasionally checked by the serial dilution method. There was a distinct difference in the growth characteristics and the penicillin producing power of these strains. Two strains (NRRL 1249 B20 and W & B) were found to be the best. Growth of these strains at 28°C. and 24°C. were tried and the latter temperature was found to be the best. A number of modifications in the medium were tried. Jaggery from the local market was found to give better results than glucose in the medium. Addition of corn steep liquor further increased the titre. The best titre obtained in the surface growths in Haffkine bottles was about 20 units per c.cm. of the medium.

By growing the mould on bran soaked in the medium in bottles, good yields of penicillin were obtained. After 7 days upto 80 units of penicillin per c.cm. of the squeezed out fluid were obtained. Each bottle yielded about 20,000 units of penicillin. By growing the mould in a large number of bottles some pure sodium penicillin was obtained following the concentration method of the Oxford workers. At present adsorption methods of concentration are being tried.

47. Enquiry on *clonorchis sinensis*, the Chinese liver fluke in the army under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

The object of this enquiry was to determine whether eggs of *Clonorchis sinensis*, the Chinese liver-fluke can develop in the common Indian snails and thus establish infestation in India, as a large number of Chinese troops were stationed during the war in this country.

The snail hosts of this fluke in China belong to the families *Bithyniidae* and *Melaniidae*. As these two families are well represented in India, transmission experiments were carried out on these snails and also other available snails.

The eggs of *Clonorchis sinensis* were collected from the faeces and bile of infected Chinese troops.

One hundred and thirty snails were exposed to the eggs, the genus distribution of the snails being: *Bithynia putchella* (66), *Melanoides lineatus* (31), *Melanoides tuberculatus* (4), *Indoplanorbis exustus* (11), *Gyraulus convexiusculus* (11), *Lymnoea luteola* (5), *Vivipara bengalensis* (2). The number in brackets indicates the number of snails exposed.

The snails were observed for cercariae for three months; no cercariae were seen and no sporocysts or rediae found on dissecting the snails.

The work could not be continued as further material from the troops was not available.

REPORTS OF ADVISORY COMMITTEES

REPORT OF THE CHOLERA ADVISORY COMMITTEE, JUNE, 1945.

The Committee met at 10-30 a.m. on Saturday, 9th June, 1945, in the Office of the Public Health Commissioner with the Government of India, Kennedy House, Simla.

PRESENT.

Lieut.-Colonel H. W. Mulligan. (*Chairman*).
Lieut.-Colonel C. A. Bozman.
Dr. B. B. Dikshit.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Lieut.-Colonel C. L. Pasricha.
Lieut.-Colonel S. S. Sokhey.
Dr. K. C. K. E. Raja.
Major M. L. Ahuja, Dr. K. V. Venkatraman } (*Joint Secretaries.*)

1. At the previous meeting of the Committee held in New Delhi on 27th November, 1944, the future of cholera research in India was discussed in general terms, and it was agreed that before the next meeting, each member of the Committee should prepare a note embodying his views on this subject. This had been done, and the notes had been circulated to all concerned. The purpose of the present meeting was to consider the proposals for cholera research put forward in this way, and to formulate tentative plans for the conduct of those researches which received the general approval of the Committee.

It was realised that it was impracticable, at the present time, to attempt work on these various problems on an ambitious scale, owing to shortage of trained personnel of the required type. It was felt, however, that some work should be commenced as soon as possible so that, when suitable personnel again became available, it would be possible to expand and extend these investigations without delay.

2. The Committee considered in detail the various proposals put forward for cholera research and suggested that preliminary investigations should be undertaken on the following problems at the institutes indicated. It was agreed that, where necessary, specific proposals should be put forward for properly constituted enquiries, to be financed by the Indian Research Fund Association, before the regular meeting of the Advisory Committee in November or December next.

I. BIOCHEMICAL INVESTIGATIONS.

(a) The role of vibrio polysaccharides and proteins in cholera immunity reactions by Dr. D. L. Shrivastava at the Central Research Institute, Kasauli.

(b) Qualitative and quantitative analyses of vibrio polysaccharides derived from vibrios grown on various culture media by Dr. D. L. Shrivastava at the Central Research Institute, Kasauli.

(c) Study of precipitin reactions in vibrios by Dr. D. L. Shrivastava at the Central Research Institute, Kasauli.

(d) Biochemistry of dehydration in cholera cases, with particular reference to the behaviour of electrolytes, by Lieut.-Colonel C. L. Pasricha at the School of Tropical Medicine, Calcutta, and Dr. K. V. Venkatraman at the King Institute, Guindy.

(e) Studies on the absorption and excretion of "sulpha" drugs, and on the concentration of these drugs in the intestine, by Lt.-Colonel. C. L. Pasricha at the School of Tropical Medicine, Calcutta, and Dr. K. V. Venkatraman at the King Institute, Guindy.

(f) Investigation on the production of "sulpha" or analogous compounds for the specific treatment of cholera, by Dr. B. B. Dikshit at the Haffkine Institute, Bombay.

II. Immunological investigations.

Directors of Institutes undertaking the manufacture of cholera vaccine agreed to investigate the following problems:

(a) Methods of assessing the antigenic efficacy of cholera vaccine.

(b) Methods of preparing simpler and more effective cholera vaccine.

(c) Criteria for the selection of vibrio strains for the manufacture of cholera vaccine, including the advisability of using one or more sub-types of *V. cholerae.*

(d) The incidence and etiology of delayed reactions following cholera vaccination.

(e) Search for a suitable animal for cholera immunity experiments.

The Committee also recommended the continuance of the work now in progress at the Central Research Institute, Kasauli, to determine the duration of immunity after cholera vaccination.

III. Basic research.

The Committee recommended that the following investigations should be initiated by Dr. S. R. Pandit at the Pasteur Institute, Shillong.

(a) Variations in the characters of El Tor vibrios and their possible role in the etiology of cholera.

(b) Variations in the characters of *V. cholerae* under the influence of bacteriophage.

(c) Study of the possible occurrence of a filterable form of *V. cholerae.*

IV. Epidemiological studies.

(i) The Committee recommended that the Director, King Institute, Guindy, should continue studies on:

(a) The protective value of anti-cholera vaccine in human herds.

(b) The persistence of cholera vibrios in convalescents, healthy contacts and waters.

(ii) The Committee recommended that the delimitation of areas of cholera endemicity in this country should, if possible, be continued by Dr. K. C. K. E. Raja.

(iii) Dr. K. V. Venkatraman and Dr. S. R. Pandit were requested to prepare notes for circulation to members on the significance of the changes in the prevalence of sub-types of *V. cholerae* during epidemics in Madras and Bengal, respectively.

V. THERAPEUTIC INVESTIGATIONS.

The Committee recommended the continuance of the cholera treatment centres recently established at Calcutta and Madras under Lieut.-Colonel C. L. Pasricha and Dr. C. G. Pandit respectively to investigate :

(a) The value of " sulpha " drugs in the treatment of cholera.

(b) The efficacy of intravenous saline therapy as at present practised.

VI. PUBLIC HEALTH MEASURES.

It was recommended that the Director, All-India Institute of Hygiene, Calcutta, should be requested to undertake research work on the following problems :

(a) Inexpensive methods for the provision of safe water supplies in areas of cholera endemicity in Bengal.

(b) Improved methods for the isolation of cholera vibrios from stools and waters.

3. Lt.-Col. S. S. Sokhey, presented a summary of the results obtained in mice on the immunising value of cholera vaccine prepared in a fluid casein hydrolysate medium. After considerable discussion, the Committee resolved that, before this vaccine was submitted to field trials, Lt.-Col. Sokhey's results should be confirmed at the Central Research Institute, Kasauli, King Institute, Guindy, and the Pasteur Institute, Shillong. Lieut-Colonel Sokhey undertook to supply samples of his vaccine and the full protocols of his experiments to the Director of these Institutes. The Committee approved Lt.-Colonel Sokhey's suggestion that Dr. Venkatraman should go to the Haffkine Institute, Bombay, to study the experimental procedures employed there, provided his services could be made available.

4. The Committee noted the recent wide publicity given in the Indian Press to a cholera vaccine prepared in America for which extravagant claims had been made. In the absence of adequate data published in scientific journals, they were unable to express any definite opinion on the efficacy of this vaccine. The Committee expressed the desirability of subjecting this vaccine to tests in various Institutes in India, and recommended that the Secretary of the Indian Research Fund Association should procure samples for this purpose.

5. The Committee considered the advisability of including Ogawa and Inaba strains of *V. cholerae* in the preparation of cholera vaccine. In view of the wide prevalence of the Ogawa sub-type of *V. cholerae* in epidemics in India at the present time, it was thought desirable to include both Inaba and Ogawa strains in the vaccine until more information on this subject had been elicited.

6. The Committee noted that a Clinical Research Advisory Committee had recently been constituted by the Indian Research Fund Association, and desired that the attention of this newly formed Committee should be directed to certain aspects of clinical research in cholera which the Cholera Advisory Committee had recommended, such as the enquiry on the biochemistry of dehydration in cholera cases, and the value of " sulpha " drugs in the treatment

of cholera. The Committee expressed the hope that in regard to researches of this kind, liaison would be maintained between the two Advisory Committees, and the Secretary of the Indian Research Fund Association was requested to convey these views to the Clinical Research Advisory Committee.

7. The attention of the Committee was drawn to the resolution passed at its last meeting regarding the use of bacteriophage in the prevention and treatment of cholera. In the absence of any fresh data on the subject, the Committee agreed that there was no case for revising the recommendations made in this regard at the last meeting.

8. During the discussions, an interesting account of the genesis of a cholera epidemic in a town in Burma isolated by floods was given by Lieut.-Colonel C. A. Bozman. The Committee recommended that Lieut.-Col. Bozman should prepare a note for publication on this unique natural experiment.

REPORT OF THE CHOLERA ADVISORY COMMITTEE, NOVEMBER, 1945.

The Committee met at 10 A.M. on Tuesday, 27th November, 1945, at the Imperial Secretariat, New Delhi.

PRESENT.

Lieut.-Colonel H. W. Mulligan. (*Chairman*).
Dr. B. B. Dikshit.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Lieut.-Colonel C. L. Pasricha.
Lieut.-Colonel S. S. Sokhey.
Dr. K. V. Venkatraman.
Lieut.-Colonel M. L. Ahuja. } (*Joint Secretaries*).

The following also attended :—
Major T. D. Ahmad.
Dr. M. C. Balfour.
Lieut.-Colonel A. N. Chopra.
Bt.-Colonel Sir Ram Nath Chopra.
Major A. N. Duggal.
Dr. P. V. George.
Dr. A. N. Goyle.
Major B. N. Khan.
Dr. V. R. Khanolkar.
Lieut.-Colonel C. K. Lakshmanan.
Major C. Mani.
Major T. C. Puri.
Dr. S. R. Savoor.

1. The Committee considered the work carried out by the following enquiries under the Indian Research Fund Association :—

(a) *Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta.*

The Committee noted with satisfaction that sulphaguanidine had proved of definite therapeutic value in a series of over 900 treated and control cases of cholera in Calcutta. Lieut.-Colonel Pasricha described the conditions under which these cases had been treated and pointed out that in many instances, where the cases had to be treated in emergency hospitals, the conditions of treatment approximated closely to field conditions. He wished to repeat the work already done on a larger series of cases both in hospitals and in the field. The Committee stressed the desirability of testing the value of sulphaguanidine and similar drugs under field conditions.

In view of the difference in the conditions under which patients in the series under report had been treated, the Committee emphasised the necessity for maintaining careful records of the conditions of treatment so that the results would ultimately be capable of analysis with reference to the conditions of treatment.

The Committee recommended the continuation of this enquiry.

(b) *Cholera treatment enquiry under the Director, King Institute, Guindy.*

Dr. C. G. Pandit explained that although the personnel for the enquiry had been recruited and trained it had not been so far possible to commence work owing to the complete absence of cholera cases in Madras. The Committee recommended that the unit should remain prepared to take up its work as soon as cases become available.

In regard to both of the above enquiries, the Committee recommended that each should take advantage of the occurrence of cholera epidemics in any part of India where conditions were considered to be favourable for the testing of drugs. The Committee considered that it would be necessary to increase the budget provision of both of the above enquiries to defray the cost of moving to and from areas suitable for their work. The selection of these areas was a matter which could safely be left to the discretion of the officers in charge of the enquiries; Bihar and Orissa were probable places for work of this kind.

(c) *Preparation of monospecific high titre cholera sera under the Director, King Institute, Guindy.*

This work has only recently been started owing to difficulties over personnel, but progress is now being made. The Committee recommended continuation of this work.

(d) *Enquiry on the immuno-chemical behaviour of V. cholerae under the Director, Central Research Institute, Kasauli.*

This small enquiry had been started in July 1945 and only work of a preliminary nature had so far been undertaken. Lieut.-Colonel Mulligan explained that it was not proposed to continue this enquiry after the end of the current financial year; the investigations at present being carried out would be taken over by the proposed new enquiry at the Research Institute if this was sanctioned.

(e) *Enquiry to test the validity of the data collected by the I.R.F.A. enquiry into the statistical evaluation of anti-cholera inoculation under the Director of Public Health, Madras and Dr. Chandrasekaran of All-India Institute of Hygiene & Public Health, Calcutta.*

The Committee considered the interim reports from the Director of Public Health, Madras, and Dr. Chandrasekaran. The Committee noted that the enquiry would terminate at the end of the current financial year and stressed the importance of the work of this enquiry being directed specifically to an investigation of the reliability of the basic data on which the conclusions reached in Dr. Adiseshan's report had been based.

2. The Committee considered the proposal for a new Cholera Enquiry to be carried out under the Director, Central Research Institute, Kasauli. The proposed lines of work of this enquiry were in close agreement with the recommendations made by the Cholera Advisory Committee at the meeting held in Simla on 9th June 1945. The Committee recommended that this work should be undertaken on the lines proposed.

3. The Committee heard with interest an account given by Lieut.-Colonel Sokhey of the progress which had been made in the preparation and testing of a new type of cholera vaccine prepared in liquid casein hydrolysate medium.

The Committee also considered a report on this vaccine by Dr. K. V. Venkatraman who, on the suggestion of the Committee, had been deputed to make a special study of this subject at the Haffkine Institute, Bombay (Dr. Venkatraman's report is attached as appendix I). The Committee decided that before this vaccine was submitted to extended field trials, Lieut.-Colonel Sokhey's work should be repeated and his results confirmed at the Central Research Institute, Kasauli; King Institute, Guindy; and the Pasteur Institute, Shillong. Lieut.-Colonel Sokhey agreed to make samples of his vaccine and the full protocols of his experiments available to the Directors of these Institutes immediately. Lieut.-Colonel Sokhey also undertook to supply a sufficient quantity of his new vaccine to Lieut.-Colonel Pasricha who agreed to observe the immediate and delayed reactions produced by the use of this vaccine in human subjects in Calcutta.

4. The Committee noted that a sample of approximately 2 litres of B.R.F. Direct Cholera Vaccine manufactured in America had been received at the Central Research Institute, Kasauli, in response to a request from the Secretary, Indian Research Fund Association. In accordance with the decision reached at the previous meeting it was decided that this vaccine should be made available to the Directors, Central Research Institute, Kasauli; King Institute, Guindy; Haffkine Institute, Bombay; and Pasteur Institute, Shillong, for laboratory tests. The Committee considered that this vaccine might suitably be tested in parallel with the new Haffkine Institute vaccine and the agar-grown vaccine at present in common use in India.

5. The Committee considered reports by Dr. S. R. Pandit and Dr. K. V. Venkatraman on the prevalence of Inaba and Ogawa sub-types of *V. cholerae* in Bengal and Assam, and in Madras, respectively (These two reports are attached as appendices II & III).

6. Dr. C. G. Pandit suggested that advantage should be taken of any epidemic of cholera which may occur in Madras in the near future to investigate the occurrence of cholera toxins. He suggested that the services of Dr. B. B. Dikshit, who had done some preliminary work on the subject, might be made available. Dr. Pandit's proposal was favourably received by the Committee. Lieut.-Colonel Sokhey considered that it would be possible to spare Dr. Dikshit for a short period to initiate the work in Madras and subsequently to continue his investigations at the Haffkine Institute on materials supplied from Madras.

APPENDIX I

A Note on the Preparation of a New Cholera Vaccine and a Method of testing the potency of Cholera Vaccines.

By

DR. K. V. VENKATRAMAN.

The new cholera vaccine of the Haffkine Institute consists of a growth of *Vibrio Cholerae* in a liquid medium containing acid casein hydrolysate, killed with formalin and preserved in phenyl mercuric nitrate. The vaccine contains both Inaba and Ogawa types of *V. Cholerae* and the strains used have been selected on the basis of their virulence to mice. The virulence of the cultures has been maintained by preserving them in a lyophilised form. Bulk production of the medium is made in large steam jacketted glass lined kettles of which the Institute has a liberal supply. The finished medium gives a negative Biuret reaction and is said not to cause any flare reaction when injected intradermally in the human skin. When dispensed in 500 ml. quantities in flat bottomed Haffkine flasks an optimal surface and depth of the medium is said to be obtained for the profuse growth of the vibrios. Col. Sokhey has recently had the shape of the flasks modified to permit of their taking one litre of the medium and it should help greatly in the large scale production of the vaccine. The organisms are grown in this medium for three days at 37°C. during which period the medium is supposed to be more or less completely exhausted. A considerable amount of lysis of the vibrios takes place during the prolonged cultivation; the lysed vibrios and the matebolites thrown off during growth are retained in the vaccine. The growth is killed by the addition of 0·1 % formalin with which it is left in contact for 7 days at 37°C. when a certain amount of detoxification also probably occurs. A proposal to increase this period to two weeks is under consideration. Finally 0·15 mg. per cent of phenyl mercuric nitrate is added as a preservative. Test of purity of the inoculum and of the growth in culture flasks and tests of sterility of the vaccine are carried out on conventional lines. A count of the organisms in one of the batches of the vaccine made in a Petroff Hauser chamber gave a value of 3000 million per ml. For reasons already stated this figure does not represent the total vibrio content of the vaccine; there is at present no means of ascertaining exactly how much of lysed vibrio matter the vaccine contains.

2. A biological test of potency of the vaccine using mice for the tests has been developed as an experimental study. In these tests groups of mice given varying doses of the vaccine in two spaced injections are challenged subsequently with approximately 10 minimum lethal doses of a virulent test culture, and an estimate of the protective dose of the vaccine reached by the 50% mortality end-point of Reed and Muench. The assumption behind the test is that the level of immunity induced in mice by two spaced injections of the vaccine determined by their resistance to a stated challenge dose is proportional to the quantity of the vaccine administered. The test thus aims to be more than a simple biological test of potency for the release of the vaccine; it is an attempt at a quantitative assay of the antigenicity of cholera vaccine by "*in-vivo*" titration.

3. The following points in the technique of the tests may be noted.

(i) Mice from a herd known to be susceptible to the action of *V. Cholerae* are selected at three weeks of age. In most of the work done up to the end of August 1945, the Haffkine Institute in-bred strain of mice were used. These required approximately a million organism (*V. Cholerae*) held in 5% mucin suspension to kill them regularly. In some later experiments a herd of swiss

mice kept as a separate colony at the Institute was found to be more susceptible ; approximately 8000 vibrios of the usual test culture being sufficient to kill them regularly.

(ii) Vaccine. The vaccine is administered subcutaneously in two doses at a week's interval between each. The vaccine dilutions are made in sterile normal saline in such a manner that the desired dose is contained in 0·2 ml. The actual doses used here have been infinitely smaller than those recorded in previous work of a similar nature.

(iii) The challenge dose is given one week after the second dose of the vaccine and consists of a suitable dilution of a 3 hour old broth culture of a known virulent strain of *V. Cholerae*, contained in 0·5 ml. of a 5 % mucin suspension. It is administered *intraperitoneally*. Plate cultures of suitable dilutions of the broth culture are put up to determine the number of viable organisms contained in the challenge dose. The uniformity in the virulence of the test cultures has been obtained by making fresh subcultures for each test from the same batch of lyophilised cultures.

(iv) The mice are observed for three days and the deaths in each group and among control unvaccinated animals are recorded. Most of the deaths occur overnight.

(v) In most of the work recorded the number of mice in each group receiving the same dose has been 5 and the variations in the dosage of vaccine have been two fold. The dosage schedule is so arranged that the 50% survival occurs near the central group.

4. In repeated tests with the same vaccine carried out on different days, closely similar values are obtained for the calculated 50% end point protective dose. A summary of the finding in 5 tests on a batch of vaccine 76-B carried out during my stay are given in table I. These tests were again repeated with doses of vaccine varying over a wider range on three separate occasions. The value of the protective dose thus obtained also approximates closely with the results obtained in the previous 5 experiments, where a closer spaced dosage schedule was adopted (Table II). With a view no doubt, to economy in the use of animals the size of the group receiving the same dose has been kept small at 5 mice in each, a greater degree of accuracy and reliability of the test will accrue if this number is increased.

Comment.

5. For many years mice have been used extensively in the determination of the virulence of bacteria and the protective value of bacterial vaccines. The fact that gastric mucin greatly enhances the virulence of many human pathogenic organisms for mice has made it possible to use relatively smaller number of organisms for these tests and render the determinations more accurate.

6. Griffitts (1942) of the U.S. Public Health Service has shown that relatively small numbers of *V. Cholerae* proliferate rapidly and set up a fatal infection in mice when injected intraperitoneally in 5% mucin suspension. He (Griffitts, 1944) has also shown that human volunteers inocluated with cholera vaccine develop mouse protective antibodies in their blood. In his opinion mice are of definite value in demonstrating specific protective substances in serum against cholera organisms. Linton and Jenkins (1944) of the Biochemical Research Foundation have used the mouse protective power of the serum of inoculated individuals to assess the value of cholera vaccine. A more direct test by determining the resistance of inoculated mice has been proposed by

Ranta and Dalman (1944) of the Connaught laboratories. They have recommended as an arbitrary standard of antigenic potency, a test employing two spaced doses of vaccine requiring survival of 100% of a group of not less than 15 mice challenged with 5 minimal lethal doses of mucinized vibrios and at least 80% of a group challenged with 10 minimal lethal doses.

7. Col. Sokhey's work is a step forward in that the test proposed provides a method of assaying the antigenicity of cholera vaccines. The extent to which results obtained with mice relate to human infection and immunity has still to remain largely a matter of conjecture and final proof must await controlled experiments in the field. This, however, does not detract from the value of the test as a laboratory means of comparing various types of cholera vaccine. The advantages of a quantitative method of assay of antigenicity in the control of the several factors involved in vaccine productions are obvious, and there is a wide field for the adoption of this test in all laboratories engaged on studies directed towards the improvement of cholera vaccine.

8. From the protocols of experiments previously carried out, it is seen that the calculated protective dose of an agar vaccine prepared from the same strain of *V. Cholerae* is about 10 times higher than the protective dose of the liquid medium vaccine i.e. under the conditions of the experiment the liquid medium vaccine appears to be a better immunizing agent. It does not necessarily follow however that when the vaccines are administered in maximum tolerated doses this difference would still persist. Further, certain differences in the production of the two types used in these experiments, other than the method of cultivation, render it desirable that these should be repeated under more comparable conditions.

9. The incidence and severity of the reactions following inoculation with the new vaccine appear to have been greater than has been the experience with the standard agar vaccine used in the province during recent years; this has been especially so with regard to the delayed reaction which occurs 8 days after inoculation. The cause of this has to be investigated and attempts made to eliminate the reaction producing factor without impairing the antigenicity of the vaccine, before it can supersede the standard agar vaccine in mass inoculation campaigns.

10. With suitable equipment the new vaccine could be more easily prepared in larger quantities and it is probable that the cost of production would be less than the standard agar vaccine; but I have not gone into the question of costs in any detail, at this stage. What is presently required is that the new vaccine which under laboratory conditions appears to be a better immunizing agent should be submitted to controlled field trials after further confirmation of its antigenic superiority is obtained and the reactions following inoculation are at least reduced by suitable modifications of the methods of production.

TABLE I.

Cholera vaccine 76 (b)

Dose in ml. test No.	·000003	·0000075	·000015	·0003	·00006	·000125	No. of organisms in test dose	Control	Calculated 50% end point protective dose
1.	1/5	2/5	1/5	3/5	3/5	4/5	7·8 millions	0/5	·000026 ml.
2.	0/5	0/5	2/5	2/5	3/5	3/5	10·4 millions	0/5	·000045 ml.
3.	1/5	1/5	2/5	2/5	3/5	3/5	10·7 millions	0/5	·000027 ml.
4.	3/5	2/5	2/5	2/5	4/5	4/5	8·6 millions	0/5	·000018 ml.
5.	2/5	2/5	3/5	3/5	4/5	4/5	7·8 millions	0/5	·000015 ml.

Numerator : No. of animals surviving.
Denominator : No. of animals used.

TABLE II

Cholera vaccine 76

Dose in ml test	−8 25×10 cc	−7 25×10 cc	−6 25×10 cc	−5 25×10 cc	−4 25×10 cc	No. of organisms in test dose	Control	Calculated 50% end point protective dose
1.	0/5	1/5	3/5	4/5	4/5	$8·6\times10^6$	0/5	·000025 ml.
	0/5	0/5	2/5	3/5	5/5	$7·8\times10^6$	0/5	·00008 ml.
*3.	1/5	1/5	2/5	3/5	5/5	88,500	0/8	·000035 ml.

*Swiss mice.

APPENDIX II

Prevalence of the two sub-types of V. cholerae in Bengal and Assam.

From records of typing available, the table below has been drawn, giving the distribution of *V. cholerae* under the two sub-types. The Assam figures relate to the period 1935–1945 and of Bengal to the period June 1939 to December 1941, being the findings of the Cholera Field Enquiry in Bengal. In this enquiry, little attention was paid for some time to the sub-typing of strains, the main tests applied being the agglutinability of the strains to a non-differential serum and whether they were haemolytic or non-haemolytic. The same may be said of a number of strains isolated in Assam. Such untyped strains do not of course find a place in the table.

For convenience, the serological sub-types of El Tor strains isolated from Waters, have also been included in the table.

It would appear from the figures that, generally speaking and for the periods mentioned, both the sub-types were found in Bengal and Assam Ogawa being more prevalent in Bengal and Inaba in Assam. Mixed infection in cases is not uncommon; in 7% of the Calcutta positive cases both sub-types were isolated. Such mixed infections might easily have been missed, if the procedure of isolating at least three discrete colonies from each case had not been adopted.

	Province	Year	Area	Number positive	Inaba	Ogawa	Remarks
Cases	Bengal	Year ending May, 1940	Khulna	51	19	32	
	,,	1941	Calcutta	284	77	187	In the remaining 20 (7%) both sub-types were isolated
	Assam	1935	Manipur	34	34		
	,,	1936	Sylhet	15	4	1	
	,,	,,	Shillong	1	1	..	
	,,	1938	,,	1	1	..	
	,,	,,	Dhubri	5	5	..	
	,,	1942	Shillong	2	1	1	
	,,	1943	,,	2	1	1	
	,,	1945	,,	1	1	..	
Water	Bengal	Year ending May 1940	Khulna	17	7	10	*V. cholerae* from water sources.
	Bengal	,,	,,	55	8	47	El Tor strains from water sources.

APPENDIX III

A Note on the Distribution of the types of V. cholerae in Madras Province.

The existence of two distinct serological types in vibrio cholerae was noted by earlier Japanese workers and confirmed by Gardner and Venkatraman (1935). These type-differences were in no way confined to the Japanese vibrios, but occurred in every large collection from several countries which they examined. Opinion was reserved on the subject of the stability of these two variants. Subsequent observations extending over a period of several years have shown that these types are stable.

It has been the practice at this Institute since 1936 to type all strains of Vibrio cholerae isolated in the course of the routine work and correlate the findings with the geographical area of origin of the strains. Early in 1936, it was noticed that of the then existing collection of Vibrio strains in the laboratory, a preponderent majority were of INABA type and there were only 4 strains of OGAWA which were derived from Madras City (2) and Ganjam (2). An outbreak of cholera in Periyakulam in 1936 attracted notice on account of vibrios being all of OGAWA type (Venkatraman and Pandit, 1937). From an examination of a large number of strains of Vibrio cholerae, originating from the several districts of the Province, it was noted in 1937 that there was a clear demarcation of the areas of prevalence of the two different types. INABA type was prevalent throughout the Northern Circars and to a great extent in the Ceded Districts. OGAWA type was prevalent in the Southern Districts.

In the west central group of districts where OGAWA was the predominant type, there was a small nest from where only INABA strains were obtained. Further observations in succeeding years were to confirm in general this distinctive distribution of the two types of vibrio cholerae until 1939 when the incidence of cholera in the province had declined to the lowest ever recorded. Subsequent to this period, a decline in the incidence of the occurrence of INABA type of vibrios has been noticed and during the widespread epidemic of 1942-1943, OGAWA vibrios were almost exclusively prevalent throughout the Province, and INABA isolations have been very few. The accompanying Table shows the number of strains of vibrio cholerae of each type isolated in the Institute from the different regions of the Province during each year from 1936 onwards. It has to be stated, however, that in only a small proportion of the recorded attacks of cholera was a bacteriological diagnosis attempted and isolations made, and that this proportion has been very variable in different districts and at different periods. The recorded incidence of cholera in these regions during the same periods is shown in the appropriate columns for purposes of comparison. Still at the time the examinations were made, the uniformity of the occurrence of one particular type only from the same area was most striking. Also in a small series of cases where repeated isolations were made from the same patient, the type of vibrio has remained constant.

TABLE

Region	1936		1937		1938		1939		1940		1941		1942		1943		1944	
	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa
Northern Circars	58 (93·5)	4 (6·5)	106 (98·2)	2 (1·8)	102 (99·0)	1 (1·0) 4451	0	0 269	22 (8·4)	241 (91·6) 3288	1 (0·9)	115 (99·1) 4499	0	154 (100) 1620	0	75 (100) 9447	0	176 (100) 11,657
Ceded Districts	18 (56·3)	14 (43·7)	87 (89·7)	10 (10·3)	60 (98·4)	1 (1·6) 3861	0	16 (100) 107	0	8 (100) 158	0	17 (100) 270	0	13 (100) 13694	3 (18·8)	13 (81·2) 7592	0	8 (100) 1,817
East Central	59 (15·9)	312 (84·1)	952 (88·2)	127 (11·8)	91 (98·9)	1 (1·1) 1569	0	3 (100) 65	0	2 (100) 68	0	3 (100) 122	7 (2·7)	255 (97·3) 980	17 (5·7)	282 (94·3) 27530	0	100 (100) 6066
Southern Districts	11 (4·6)	228 (95·4)	8 (4·3)	177 (95·7)	23 (67·6)	11 (32·4) 3558	0	29 (100) 1184	0	1 (100) 111	2 (8·7)	21 (91·3) 139	2 (0·9)	213 (99·1) 8285	2 (1·5)	131 (98·5) 26108	2 (4)	48 (96) 11,032
West and West Central	5 (8·1)	57 (91·9)	44 (34·4)	84 (65·6)	0	0 337	0	0 20	0	1 (100) 28	0	0 33	0	9 (100) 727	18 (66·7)	9 (33·3) 43877	0	37 (100) 4,152

( ) Figures in brackets denote the percentage of the total isolation in the region during the year.

REPORT OF THE MALARIA ADVISORY COMMITTEE

The Committee met at 2-30 p.m. on November 26, 1945. It was constituted as under :—

Major-General G. Covell (*Chairman*).
Lt.-Col. M. K. Afridi.
Dr. B. Ananthaswamy Rao.
Rai Bahadur Dr. A. C. Banerjea.
Bt.-Col. Sir Ram Nath Chopra.
Lt.-Col. H. W. Mulligan.
Dr. D. K. Viswanathan.
Lt.-Col. Jaswant Singh (*Secretary*).

The following were also present :—
Major A. K. Adhikari.
Major T. D. Ahmad.
Dr. M. C. Balfour.
Lt.-Col. C. A. Bozman.
Dr. G. Brookes.
Lt.-Col. A. N. Chopra.
Major A. N. Duggal.
Lt.-Col. C. K. Lakshmanan.
Group Capt. G. W. McAleer.
Lt.-Col. C. L. Pasricha.
Major T. C. Puri.
Major R. Senior White.

Proceedings.

I. The reports on enquiries carried out during the current year and proposals for 1946-47 were considered.

Existing enquiries.

1. *Malaria Institute of India (Research Section).*

Research activities have again been curtailed by abnormal conditions brought about by the war. Six courses in malariology have been held in 1945 at which 147 officers attended, bringing the total number trained during the war to 630.

Laboratory research has been restricted mainly to the preparation of antisera for determining the source of mosquito blood meals. A series of tests on monkey malaria have been carried out with a new ICI synthetic drug, M4888, which has shown considerable promise, being superior in certain respects to mepacrine. Two indigenous drugs were tested but were found to have no plasmodicidal effect. A new blood stain has been developed which can be used as a substitute for the standard Romanowsky stains at present in use.

A great deal of work has been done with regard to the development of DDT emulsions and equipment for use with DDT. A number of field trials with DDT have been carried out. The routine identification of mosquitoes and other insects of medical importance has been carried out as in former years,

Southern India Branch.

An extensive series of studies on the bionomics of *A. fluviatilis*, both larvae and adults, is in progress. Laboratory colonies of *A. fluviatilis*, *A. stephensi* and *Aedes albopictus* have been maintained. A strain of *P. gallinaceum* is also being maintained.

Insecticide and Mosquito Repellent Enquiry.

A series of field trials with both oily solutions and emulsions of DDT have been carried out at Marianbari, Bengal Duars. The results have demonstrated that DDT is extremely effective against *A. minimus*, the chief malaria carrier of foothills of Bengal, Assam and Burma.

Proposals for 1946-47.

(a) *Delhi.*—1. Systematic testing of insecticidal sprays, mosquito repellents and larvicides and spraying apparatus under laboratory and local field conditions.

2. Testing of the therapeutic effects and toxic action of drugs as evidenced by their effect on animal malaria as occasion demands.

3. Intensive study of bionomics of mosquito colonies maintained in the laboratory.

4. Determination of food preferences of various species of mosquitoes by the precipitin test.

5. Attempts to increase the efficiency of the various antisera employed for precipitin tests.

6. Observations on the bionomics of *A. culicifacies.*

7. Epidemiological studies on malaria conditions in certain villages and coolie camps.

(b) *Coonoor.*—1. Observations on the bionomics of *A. fluviatilis* and other mosquito species of the Nilgiris foothills.

2. Epidemiological studies in certain villages in the Nilgiris.

3. Maintenance and study of P. *gallinaceum* and mosquito colonies.

(c) *Insecticide and Mosquito Repellent Enquiry.*—1. Systematic testing of insecticidal sprays, repellents, larvicides under field conditions against various vector species in different parts of India.

2. Testing of spraying apparatus under different field conditions.

3. Observations on the bionomics of vector species of mosquitoes in different parts of India.

2. *Statistical enquiry under Dr. M. Yacob.*

This enquiry has for its object the development of methods of forecasting of epidemic malaria in the Punjab. The study of the periodicity of malaria based on the records of previous 77 years has been completed and a paper entitled, "Investigation of long term periodicity in the incidence of epidemic malaria in the Punjab" published in the June 1945 issue of the *Journal of the Malaria Institute of India.*

Proposals for 1946-47.

It is proposed to examine the cumulative effect of the spleen indices of two or more previous years on the intensity of subsequent malaria epidemics and to test the effect and evaluate the statistical significance of the relationship of sun spots on malaria.

3. *Mammalian Malaria Enquiry at the Central Research Institute, Kasauli.*

The work of this enquiry commenced on May 15th but the full complement of personnel was not available till September 20th when Dr. Lloyd (Histopathologist) and Mr. Woodward (Technician) who had been deputed by the Royal Society, arrived in India.

The object of the enquiry is to determine whether or not an exo-erythrocytic cycle occurs in mammalian malaria, a problem of primary importance in relation to the prophylaxis and treatment of malaria and the prevention of relapses.

A great deal of preliminary work has been accomplished and the ground has been cleared for future work. A very large number of smears and sections have been prepared from monkeys infected with *P. cynomolgi*. The material so far examined has yielded negative results.

Proposals for 1946-47.

It is proposed to continue the work on similar lines.

4. *Enquiry on immunology in malaria under Dr. B. M. Das Gupta.*

Immunological relationship between the *P. knowlesi* and the different species of human malaria in human hosts and between the strains of human species from different localities have been studied, and the results show that *P. knowlesi* infection confers no immunity against the human species of *Plasmodia*, and that local and other Bengal strains of *P. vivax* are probably homologous.

Proposals for 1946-47.

It is proposed to continue work on the same lines with different strains of parasite obtained from different localities.

5. *Filariasis enquiry under Dr. Sundar Rao.*

A demonstration project involving the removal of Pistia from ponds to prevent the breeding of *mansonioides*, the carrier of *W. malayi* infections, was carried out at Ratanpur in C.P.

A survey was carried out in the village of Narkher in the C.P., where the infection is solely due to *W. bancrofti*.

Observations in Cuttack showed that local conditions as regards the prevalence of filariasis and of Culex mosquitoes remain the same as in 1922 and 1930.

A study of microfilariae from Ratanpur (*W. malayi*) and Narkher (*W. bancrofti*) revealed no morphological differences.

Studies in the pathology of filarial infection have revealed no new factors.

A number of drugs were tested for their therapeutic value including penicillin. Of these anthiomaline proved helpful (though toxic) in the control of lymphangitis. Mapharside cleared up the urine in one case of chyluria,

Filarsen was thought to improve the general tone of the patients, thus helping them to overcome the susceptibility to attacks of lymphangitis. No drug had any effect on the parasites in the tissues.

Proposals for 1946-47.

1. Intensive field surveys in endemic areas.
2. Trial of certain synthetic drugs.
3. Control demonstration projects in selected areas.

PROPOSALS FOR NEW ENQUIRIES.

1. *Blackwater fever enquiry under Captain Inderjit Singh.*

The action of antivenene on haemolysis is to be further studied and the reactions of the uterus recorded.

2. *Enquiry on the chemotherapy of filarial infection under Dr. N. N. De.*

It is proposed to synthesise a number of organic compounds. These will be first tried *in vitro* and if found active will then be tested on naturally infected lizards.

3. *Enquiry on adult A. stephensi under the Director, School of Tropical Medicine, Calcutta..*

It is proposed to attempt to determine the race of *A. stephensi* concerned in the transmission of malaria in Calcutta and to make an intensive study of the density, seasonal prevalence, feeding habits and day time resting places of the species.

4. *Enquiry into the control of Phlebotomus argentipes with DDT spraying under Dr. D. N. Roy.*

It is proposed to carry out investigations in certain villages in Darbhanga District where *P. argentipes* are abundant and kala-azar endemic. DDT will be used in an attempt to control sandflies in this area.

The Committee made the following recommendations :—

EXISTING ENQUIRIES.

1. (a) Malaria Institute of India (Research Section).
   (b) Malaria Institute of India (S. India Branch).
   (c) Insecticide and Mosquito Repellent Enquiry.
2. Mammalian Malaria Enquiry at the Central Research Institute.

The Committee approved the proposals put forward for the continuance of these enquiries in 1946-47.

3. Statistical enquiry under Dr. M. Yacob.
4. Enquiry on immunology in malaria under Dr. B. M. Das Gupta.

The Committee did not recommend the continuance of these enquiries.

5. Filariasis Enquiry under Dr. Sundar Rao.—The Committee recommended the continuance of this enquiry, but suggested that its programme should be examined by a sub-committee with a view to modifying its proposals. Major Senior White expressed his willingness to serve on such a committee.

PROPOSALS FOR NEW ENQUIRIES.

1. Enquiry on adult *A. stephensi* under the Director, School of Tropical Medicine, Calcutta.

2. Enquiry on the control of *Phlebotomus argentipes* with DDT spraying under Dr. D. N. Roy.

The Committee approved the proposals put forward for these enquiries.

3. Blackwater fever enquiry under Capt. Inderjit Singh.

4. Enquiry on the chemotherapy of filarial infection under Dr. N. N. De.

The Committee did not recommend these proposals.

II. The Committee considered a letter from Prof. Subrahmanyan, Indian Institute of Science, Bangalore, suggesting that an investigation should be undertaken regarding the toxic effects of mepacrine in cases of malnutrition. The Committee did not support this proposal.

REPORT OF THE THIRTEENTH MEETING OF THE NUTRITION ADVISORY COMMITTEE OF THE INDIAN RESEARCH FUND ASSOCIATION HELD IN NEW DELHI, ON THE 28th NOVEMBER, 1945.

COMPOSITION OF THE COMMITTEE.

PRESENT:

Lieut.-Colonel C. A. Bozman, I.M.S., Public Health Commissioner with the Government of India (*Chairman*).

Professor S. P. Agharkar, Professor of Botany and Head of the Botany Department, University College of Science, Calcutta.

Dr. K. P. Basu, Reader in Chemistry, and In-charge of the Biochemical Laboratory, University of Dacca, Dacca.

Khan Bahadur Dr. A. H. Butt, Director of Public Health, Punjab, Lahore.

Dr. B. C. Guha, Chief Technical Adviser to the Government of India, Department of Food, New Delhi.

Dr. V. N. Patwardhan, Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Dr. G. Sankaran, Professor of Biochemistry and Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

Sir Theodore Gregory, Economic Adviser to the Government of India, Mr. John Sargent, Secretary, Education Department, Government of India, Mr. H. R. Stewart, C.I.E., Vice-Chairman, Imperial Council of Agricultural Research, Major G. Williamson, O.B.E., Animal Husbandry Commissioner with the Government of India, and Dr. W. R. Aykroyd, C.B.E., M.D., Sc.D., Director, Nutrition Research Laboratories, Coonoor, were unable to be present.

The following were present by invitation:—

Dr. Bashir Ahmad, Assistant Director, Council of Scientific and Industrial Research, University Buildings, Delhi.

Dr. S. Ahmad, Nutrition Officer, Public Health Department, Bihar, Patna.

Dr. (Miss) Kamala Bhagvat, Assistant Director, Nutrition Research Laboratories, Coonoor.

Dr. R. E. Dadachanji, Nutrition Officer, Finance Department (Supply), Haffkine Institute, Bombay.

Dr. M. B. Daver, Nutrition Officer, Medical and Public Health Department, Hyderabad (Deccan).

Dr. S. S. De, Lecturer on Food Technology, Indian Institute of Science, Bangalore.

Dr. P. V. George, Nutrition Officer, Public Health Department, Madras.

Mr. T. Gonsalves, Assistant Secretary, Indian Research Fund Association.

Mr. W. H. Kirby, Rationing Adviser to the Government of India, Department of Food, New Delhi.

Dr. K. Mitra, M.B.E., Deputy Director Rationing (Nutrition), Government of India, Department of Food, New Delhi.

Dr. M. V. Radhakrishna Rao, Assistant Director, Department of Nutrition and Experimental Pathology, Haffkine Institute, Parel, Bombay.

Dr. D. M. Roy, Nutrition Officer, Public Health Department, Central Provinces, Nagpur.

Lieut.-Colonel B. H. Smith, I.M.S., Assistant Director (Nutrition), Medical Directorate, Delhi.

Professor V. S. Subraymanyan, Professor of Biochemistry, Indian Institute of Science, Bangalore.

1. TO CONSIDER THE PROGRESS OF NUTRITION WORK IN PROVINCES AND STATES.

The Committee considered reports of work carried out in Madras, H.E.H. the Nizam's Dominions, Hyderabad (Deccan), Bihar, the Punjab, Bombay and the Central Provinces and Berar. A brief account of the work is given below :—

Madras : The report submitted was on work carried out for the half-year ending 31st October, 1945. Madras has only recently appointed a nutrition officer and nutrition activities have experienced the teething difficulties that so frequently happen in the setting up of a new organisation. Work has been concentrated mainly on the following subjects :

Diet surveys carried out on a regional basis ;
Enquiries into deficiency diseases ;
Biometric studies ;
Studies of vital statistics in relation to food and nutrition ;
Health education work through exhibitions and cinema shows ;
Laboratory work ;
Popularisation of the compost methods of the disposal of town refuse in small municipal towns.

Diet surveys were carried out in the deficiency districts of Bellary and Kurnool and were of the rough and ready type advocated for rapid surveys. This method was, however, soon abandoned in favour of the more classical type of enquiry. The enquiry revealed that in these areas the consumption of rice has fallen markedly while that of millets has increased, it is probable that the cost of rice and the difficulties in obtaining it in these areas of shortage, accounts for this finding. Among the pulses red gram is both largely grown and consumed in these areas. The consumption of the protective foods, e.g. green leafy vegetables, milk and its products, is almost negligible. The survey seems to establish that in these parts of Madras it has been possible for a working class population to manage to continue to live in the absence of adequate supplies of rice by utilising grains normally neglected in this area.

An important feature of the work has been the biometric studies which have been taken up in order to attempt to find out a basis of an age, weight and height standard from which the state of nutrition of children may be assessed. These biometric analyses are by no means complete and the progress of this work will be watched with considerable interest.

At the Nutrition Laboratory where the technical staff available is inadequate attempts have been made to work out certain recipes for palatable preparations of ragi and to experiment with the incorporation of food yeast into various food preparations. Material has been collected to open a nutrition museum as soon as suitable premises become available.

H.E.H. the Nizam's Dominion : The Nutrition Advisory Committee, the formation of which was taken up last year, has been functioning during the year under investigation.

During the year under review work has been concentrated mainly on school feeding schemes; on the setting up of milk booths for the distribution of milk to children and expectant mothers; on mobile canteens and on canteen feeding arrangements. In all these great difficulty has been experienced in obtaining the necessary supplies of milk and various methods are under consideration to overcome this. In the school feeding scheme it is proposed to give 8 ozs. of reconstituted milk plus seasonal fruits and vegetables and some form of carbohydrate in the form of biscuits, etc. On account of the shortage of milk distribution at the milk booths has been restricted to children below two years and pregnant and nursing mothers. In order to get over a very real difficulty which concerns the supply of milk to persons who need it but do not like to approach a public place to obtain their supply, arrangements have been made to deliver the necessary quota to them at their own houses with the levy of a small charge.

It is hoped that the mobile canteens which will be set up will serve the purpose of demonstration kitchens as well as offering to the public prepared food at cost price. It is hoped also that by selling cheap and thus attracting a large number of clients these mobile restaurants may pay their way to a great extent. Those canteens which are run in connection with industry are already popular and at least 30,000 workers in the city are utilising the facilities thus afforded. Refreshments are served during rest hours and attempts are being made to introduce a scheme whereby a mid-day meal may be made available.

The nutrition organisation of the State also carried out repeat surveys of sample groups of families in Hyderabad city and publicity and propaganda work. On the purely technical side investigations into the incidence of fluorosis have been prosecuted.

Bihar: During the year under review the nutrition officer who has been working for some years in Bihar was loaned by his Government for a Central Government appointment. His place has, however, been taken by another nutrition officer and work in this Province which was among the earliest to take up this subject has not been interrupted.

Dietary surveys were undertaken into the food intake of Oraon families, these being one of the aboriginal tribes in the district of Ranchi. The idea was to compare the results obtained in this case with those obtained from a survey completed last year of Munda families in the same district. It was found during this investigation that the nutritional state of the Oraon children as compared with the Munda children was better, due apparently to a more liberal intake of food.

On the laboratory side studies were carried on into protein metabolism with an interchange of cereals in the diets by human feeding experiments. Samples of mustard seed were analysed at the request of the Prison authorities and other samples were received from other sources.

In this Province education, propaganda and publicity work has always been prominent and this was continued as usual during the year under review.

When the Committee were considering the record of the analysis of four samples of common types of meal which were reported by the nutrition officer emphasis was laid on the necessity for having analysis of diets in families checked wherever possible by analysing cooked diets in institutions. It was recommended that the recorded results should always include a description of the method

of cooking. The actual method of sampling cooked food to be adopted in individual cases will of course have to be determined by the individual worker.

Bombay: The Government of Bombay have taken up the question of nutrition seriously and much work has been done in this Province on many items of this important problem. The distribution of milk in the city of Bombay and its suburbs is subsidised and now covers very large numbers of persons who receive this article of food at 50% concessional rate. The cost to Government for subsidising this scheme is at present over Rs. 1,20,00,000 per annum. The usual difficulties of obtaining milk supply in adequate quantities are being met partly by the importation of large quantities of separated milk powder. In addition a special farm has also been started with nearly 1,000 milk buffaloes. Other schemes which are receiving attention in the Province concern the development of more foodstuff, both protective and others, such as cereals, pulses, vegetables, fish, poultry and eggs.

The Bombay Government have started an experiment by taking over the mass feeding of unmarried personnel and recruits at the police headquarters at Thana. Each man is charged Rs. 15 per month, the balance of the expenditure being met by Government. The scheme met with some trouble in its early days as those participating were accustomed to somewhat more bulky feeds than the balanced diet provided at the community feeding centre, but it has now become popular and the police authorities have recommended to Government that similar facilities should be provided at four other centres in the Province. The clerical staff at the Secretariat, Bombay, are also being included in another community feeding experiment where at a charge of six annas per meal a fairly balanced vegetarian meal is supplied. The overhead charges of this scheme are being borne by Government. A sum of nearly rupees one lakh has been spent on the construction of a suitable building and the provision of furniture and equipment. Yet a third experiment has been instituted in a rural area in Poona. Students at various centres there can now at a cost of Rs. 10 per month join a community feeding experiment which has already caused a distinct improvement in the health of those participating. Some progress is recorded with the setting up of canteens for industrial workers, though the general circumstances of life of this class of person in Bombay make the introduction of such schemes difficult.

School feeding continues to receive attention and over 1,20,000 children attending compulsory primary education in schools are concerned. The scheme gives to those children who are reported to be below standard six ounces of milk with or without the addition of shark liver oil free of charge per day. The giving of this milk is supervised by school teachers and the Corporation has been spending nearly five lakhs of rupees on this. It should be understood that this scheme is in addition to the Government subsidised scheme referred to above. An interesting development in the feeding of school children is a proposal to introduce the giving of a tablet, prepared at the Haffkine Institute, which will contain shark liver oil, vitamin A and vitamin D. It has been found possible to make a very palatable tablet using chocolate and as soon as the necessary tableting machine arrives it is hoped that some 200,000 children will be able to receive a tablet daily.

This scheme was discussed at length by the Committee and the opinion was recorded that to meet the minimum nutrition requirements in the country it is desirable to give to those population groups that need them multi vitamin

tablets containing other important minerals. If such tablets are not readily available, Provincial Governments should consider whether it would be possible to follow the lines of the scheme now being considered by the Bombay Government. In all cases where this scheme is introduced periodic checks must be carried out on the vitamin content of the tablets distributed.

A special rapid nutrition survey carried out in Thana city revealed that the class of population most unfavourably placed from the point of view of nutrition was the clerical middle class, 48% of whom were suffering from what could be described as clinical malnutrition. The corresponding figures for other classes are 15% for the labouring class and 23·5% for the school going population. These surveys have indicated also that some 32% to 66% of the population was subject to clinical malnutrition. Other work in this connection included school surveys which seemed to indicate that enlargement of tonsils is a defect that ranks high among this class of the community.

Punjab: The Punjab Public Health Department is concerned with nutrition activities and under its auspices a survey was carried out in the winter of 1944-45 in Ambala District. In addition a diet survey lasting for a week of 170 families at four centres was also carried out. The five-year experiment on the prevention of simple goitre through using iodised salt entered its fourth year and the results, as already reported, continue to appear to be very encouraging. Various samples of foodstuffs, waters, etc. were analysed during the year. The Director of Public Health reports that nutrition work in this province is making steady though somewhat slow progress and he hopes that a scheme for establishing a nutrition unit in Kangra Valley now before Government will be sanctioned. This unit when it gets to work should considerably improve the state of health of a population known to be suffering from malnutrition.

In accordance with the recommendation of the Food Requirements Sub-Committee of the Nutrition Advisory Committee in 1944 a scheme has been submitted to Government for nutrition planning in which the annual requirements of both humans and livestock have been calculated as against the average annual production figures of various foodstuffs. In this scheme stress has been laid on the necessity for formulating a scientific food policy with particular reference to the requirements for protective foods. If approved such a policy should help to make the province self-supporting in respect of its food.

Proposals have also been submitted to expand the nutrition section of the Department of Public Health by establishing a nutrition research laboratory at provincial headquarters. As in past years publicity work through lectures, literature and posters was carried out.

Central Provinces and Berar: The Nutrition Section of the Public Health Department of this province only came into being late in April 1945 when a nutrition officer was appointed. Work has however already been started in diet surveys in both urban and rural areas and a special investigation has been undertaken regarding the various factors influencing the occurrence of lathyrism. The province is experiencing some difficulty on account of the incidence of this disease and the importance of this was realised by the Committee.

It was decided that the nutrition officer of the Central Provinces should make a special reference to those members of the Nutrition Advisory Committee who are already working on this problem in order that he might as soon as possible be in possession of the latest information and the latest views on its causation and prevention. The Committee also felt that this was a subject to

which the attention of the Clinical Research Advisory Committee should be invited.

Mr. W. H. Kirby, Rationing Adviser to the Government of India, at the conclusion of the above review, spoke on the wider aspects of food and nutrition policies. He stressed the great need for cooperation between the various authorities who are concerned in work of this nature. He expressed the thanks of his department for the facilities afforded at the Nutrition Research Laboratories, Coonoor, in the way of lectures delivered by the Director, Dr. W. R. Aykroyd, to representatives from Provinces and States who are concerned with food policy. He indicated some of the practical lines along which his department are considering how the general nutrition state of the people might be improved in the immediate post-war years and he referred to the publicity activities of the Food Department making a plea for contributions from all those working on the subject of nutrition.

2. To consider the draft of the Technical Report of the Soya Bean Sub-Committee regarding work done on Soya Bean under the I. R. F. A.

The report was accepted with a slight modification as explained in the report which is printed as Appendix I to this report.

3. To consider a note entitled "Note on the results of diet surveys in India—Part II" prepared by Dr. W. R. Aykroyd, C.B.E., M.D., Sc.D., Director, Nutrition Research Laboratories, Coonoor, at the request of the Nutrition Advisory Committee (November 1944).

The Committee considered this note and decided that Dr. Aykroyd should be asked to recast it so as to produce it in the form adopted in the note on this subject which he had prepared in 1939.

4. To consider a note regarding a paper entitled "Studies on protein, fats and mineral metabolism by Dr. K. P. Basu, D.Sc., Ph.D., Reader in Chemistry, Dacca University, Dacca.

The Committee considered that since Dr. Basu had in his note referred to all the published work on human experiments connected with this subject that have been carried out in India it should be published.

5. To consider certain documentation on 'Vitamin Enriched Rice-Roche Process' received from Messrs. Hoffman La Roche Inc. and a note on the subject prepared by Dr. W. R. Aykroyd, C.B.E., M.D., Sc.D., Director, Nutrition Research Laboratioes, Coonoor.

The Committee having considered this note, discussed the question of the fortification of rice and recommended that a Sub-Committee be appointed to investigate the question of the fortification of staple foodstuffs in all its aspects. They suggested the Sub-Committee should be composed of the following :—

Dr. Bashir Ahmad
Dr. K. P. Basu
Dr. (Miss) Kamala Bhagvat
Dr. B. C. Guha
Dr. V. N. Patwardhan
Dr. G. Sankaran

The Committee recommended that this Sub-Committee should work in the closest collaboration with the organisation in the Food Department which is considering the question of the fortification of foodstuffs.

Proceedings of the meeting of the Milk and Milk Products Committee of the I.C.A.R. held in New Delhi on the 30th October, 1945.

The Chairman reported to the Committee that he had been asked particularly to bring to their notice results obtained in a research carried out into the vitamin A content of ghee and the nutritive value of vanaspati by the Imperial Veterinary Research Institute in a report for the year 1944-45 discussed at the meeting referred to under this heading. The Chairman read to the Committee the results of this research as recorded in a paper considered by the Milk and Milk Products Committee. The Committee recorded that they heard of this with interest and recommended that further work be carried out under the auspices of the I.C.A.R. on this important subject. The Chairman stated that details of this work as available in the proceedings of the meeting of the Milk and Milk Products Committee will be circulated to members.

6. TO REVIEW WORK IN PROGRESS AND CONSIDER THE QUESTION OF CONTINUATION OR OTHERWISE OF THE ENQUIRIES.

The Committee recommended the continuation of the following enquiries:

(i) Nutrition researches under Dr. W. R. Aykroyd at the Nutrition Research Laboratories, Coonoor.

(ii) Ascorbic acid enquiry at the Univeristy College of Science and Technology, Calcutta.

(iii) Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

(iv) Nutrition research unit under Dr. K. P. Basu at Dacca University, Dacca.

(v) Nutrition research unit under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay.

(vi) Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Laboratories of the Board of Scientific and Industrial Research, University Buildings, Delhi.

NOTE.—In order to expedite the work of the enquiry the appointment of an additional worker was recommended.

(vii) Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

(viii) Enquiry on the chemical nature and nutritional availability of food iron at the University College of Science and Technology, Calcutta.

(ix) Enquiry on poor rice diets and food preparations in common use in South India under Miss D. Pearson at the Women's Christian College, Madras.

(x) Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary protein to blood and muscle haemoglobin under Professor M. Damodaran at the University Biochemical Laboratory Triplicane, Madras.

The following enquiries have already terminated or will terminate this year :—

(i) Enquiry on protein metabolism by human feeding experiments under Dr. K. Mitra at the Public Health Laboratories, Bankipur, Patna.

(ii) Enquiry into methods of treating sick starving destitutes in Calcutta based on the All-India Institute of Hygiene and Public Health, Calcutta.

(iii) Enquiry on beri beri in Cocanada under the Director, Nutrition Research Laboratories, Coonoor.

The following new enquiries were recommended :—

(i) Nutrition research unit under Dr. B. C. Guha at the University College of Science and Technology, Calcutta.

(ii) Nutrition research unit under Professor V. Subrahmanyan at the Indian Institute of Science, Bangalore.

NOTE.—In recommending the establishment of this unit the Committee pointed out that behind the idea of establishing nutrition units was the intention of so disseminating this work that local Governments should be stimulated themselves to contribute to the advancement of the science of nutrition. There was also the question of affording facilities for continued employment of existing I.R.F.A. employees.

(iii) Enquiry on the nutritive value of soya-bean milk under Dr. S.S. De at the Indian Institute of Science, Bangalore.

NOTE.—The importance of the introduction of human experiments as early as possible into the field of this enquiry was stressed.

(iv) Enquiry entitled "Preparation of anti-rachitic substances under Professor B. V. Bhide at the Maharaja Pratap Singh Chemical Laboratory, S. P. College, Poona.

The following enquiries were not recommended :—

(i) Enquiry into causes and prevention of rancidity and standardisation of common edible oils and fats under Prof. M. N. Goswami, Head of the Department of Applied Chemistry, University College of Science and Technology, Calcutta.

(ii) Enquiry on beri beri pellagra, riboflavin deficiency, post puerperal neuritis under Dr. T. K. Raman, Professor of Therapeutics, Andhra Medical College, Vizagapatam.

(iii) Enquiry on the metabolism of manganese under Mr. M. N. Rudra at the Prince of Wales Medical College, Patna.

(iv) Enquiry on biochemical changes in diabetes and other metabolic diseases in the tropics under Dr. J. P. Bose, Officer-in-charge of the Department of Biochemistry and Diabetes, School of Tropical Medicine, Calcutta.

APPENDIX I

Report of the Soya Bean Sub-Committee, November 27th, 1945.

The Sub-Committee had before them for discussion a draft report on "Soya Bean" compiled by Dr. W. R. Aykroyd on their behalf. The report was discussed at some length. A few typographical errors were corrected. Suggestions regarding (a) the removal of ambiguity of expression at some places; (b) additional references to published work; (c) recasting of some tables with the addition of fresh data and (d) the inclusion of Dr. Basu's work on soya bean milk were agreed to unanimously and consequential changes made in appropriate places. The Sub-Committee also agreed to delete the first paragraph on page 30, as it was felt that its inclusion was unnecessary.

The Sub-Committee felt that the conclusion on page 31 could be more suitably worded and agreed on the following paragraphs to be added as numbers 12 and 13 in continuity with the rest of the Chapter VII.

(12) Although soya bean contains more fat, minerals, vitamins and 'available' proteins than other pulses, it has, for some unknown reason, not proved itself superior to other pulses within the range of the experiments reported here. It is, however, possible that soya bean may, as has been pointed out elsewhere, prove a better supplement than other pulses to typical Indian diets which are quantitatively inadequate and based on cereals. Further work on this aspect is desirable.

(13) Taking the results obtained so far into consideration, the Sub-Committee is of the opinion that as a supplement to typical Indian diets based on cereals, but supplied adequate in quantity, soya bean has no special advantage over common Indian pulses.

The Sub-Committee is not in a position therefore to advocate immediately the encouragement of the production of soya bean on a wide scale in India for use as a substitute for Indian pulses. The question should, however, be reconsidered if and when further evidence on the nutritive value of soya bean becomes available.

With the above mentioned modifications the draft report was adopted by the Sub-Committee.

The Sub-Committee then heard an account from Professor V. Subrahmanyan, of the interesting work on soya bean milk which he and his colleagues have been carrying out at the Indian Institute of Science, Bangalore. The actual proposal for an enquiry will be discussed by the Nutrition Advisory Committee.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met on the 28th November, 1945, the following members being present.

Dr. V. R. Khanolkar (*Chairman*).
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Dr. A. N. Goyle.
Dr. K. V. Venkatraman.
Lieut.-Colonel S.S. Sokhey (*Secretary*).

The following were also present :

Major B. N. Khan.
Dr. P. V. George.
Dr. S. R. Savoor.
Dr. B. B. Dikshit.

The reports of the enquiries in progress during the year were considered.

1. PLAGUE ENQUIRY UNDER THE DIRECTOR, HAFFKINE INSTITUTE, BOMBAY.

(a) Plague vaccine : (i) Preparation of casein hydrolysate : Methods were described for the preparation of casein hydrolysate on a large scale. Numerous difficulties encountered were successfully met, and a medium produced which gives very good growth of *Pasterurelia postis*, and what is equally important is free from proteins and primary and secondary proteoses. The medium so prepared gives a negative biuret reaction and on intradermal injection does not produce any skin reactions. The medium is devoid of any toxic action in mice. The medium is eminently suited for the preparation of a direct plague vaccine.

(ii) Optimal period of incubation of plague cultures : Studies reported showed that in the casein hydrolysate a fully potent vaccine is obtained in two weeks' incubation at 20°C. This observation has led to the shortening of the period of incubation from 4 to 2 weeks.

(iii) Keeping qualities of casein hydrolysate plague vaccine : Exposure of the vaccine to a temperature of 37°C. for a period of 8 months showed no measureable loss of potency. The experiments are being continued and it is possible the potency of the vaccine may remain intact for much longer periods.

(iv) Preparation of a note for the Pharmacopoeial Committee: The Committee requested Lieut.-Colonel Sokhey to prepare a note on the preparation of plague vaccine and send it to the Pharmacopoeial Committee.

(b) (i) Field trials of sulphonamides and anti-plague serum in the treatment of plague : No suitable opportunity occurred during the year to undertake field trials. The Committee discussed the question of these field trials, the main object of this enquiry, and came to the conclusion that the present staff of this enquiry is not adequate for this purpose. The present personnel of the unit consists of a couple of laboratory assistants and a few laboratory attendants while the Haffkine Institute has to find the senior staff. The Committee came to the opinion that the personnel of the enquiry should be strengthened and thus a small trained nucleus created which could be sent out at a short notice without interfering with the routine duties of the Haffkine Institute. With this object in view the Committee recommend that the skeleton staff of

the enquiry should be one medical officer, grade B, on a starting pay of Rs. 360/- p.m.; one research worker (chemist) grade C, two laboratory assistants and three laboratory attendants. This staff will be strengthened by additional staff such as nurses, ward orderlies etc. when an emergency arises. The Committee is of opinion that this skeleton staff will have enough work in the Institute in testing various sulphonamides, independent of the field trials.

The Committee is of the opinion that this work should be continued during 1946-47, but considers that the personnel of this enquiry should be reorganised on the lines indicated above and Lieut.-Colonel Sokhey asked to submit an amended budget. Rough calculation showed that the budget would amount to about Rs. 18,000/-.

(ii) Memorandum on the use of Sulphonamides in the treatment of Plague.

The memorandum prepared on the subject by Lieut.-Colonel Sokhey for use of authorities engaged in the treatment of plague was approved for issue, with the modification that in the table for recording results the date of inoculation should be included.

2. Plague enquiry under the Director of Public Health, Madras.

The material collected in connection with the routine plague preventive measures in Hosur District were used for the purpose of investigation of the distribution of different species of fleas and their relation to the transmission of plague. It was noted that brasiliensis and cheopis predominated in domestic rats. Certain interesting observations were made regarding sylvetic plague but the evidence produced was not enough to establish the existence of sylvetic plague.

The Director of Public Health, Madras, does not propose to continue this enquiry, but has submitted proposals for a new enquiry, which are discussed below. Now that the Hosur enquiry has been stopped the Committee requests that the Director of Public Health be asked to draw up a note consolidating the results of five years work during which the enquiry was carried out.

3. New proposals.

Plague enquiry in the Nilgiris: The proposed research enquiry in connection with anti-plague measures in the Nilgiris which the Madras Government has planned for the next year was considered. The Madras Government proposes to put up an organisation for the prevention of plague in the Nilgiris at an annual cost of Rs. 1,05,020. The material collected during this routine work is proposed to be utilised for research work on a number of points. The Director of Public Health, has indicated that this research work will entail an expenditure of Rs. 30,000/- out of the total sum budgetted. He requests that the I.R.F.A. be pleased to make a contribution of Rs. 10,000/- towards this research expenditure as in previous years. The Committee considered this proposal very fully. The Committee was not in favour of a token grant, but recommends that a grant of Rs. 10,000/- be made for engaging special personnel or for buying equipment for specific enquiries. Further that this grant be made contingent on the Madras Government implementing the whole scheme as indicated in the letter of the Director of Public Health. With this object in view it is recommended that the Director of Public Health, be asked to submit a specific proposal.

(4) Report on the use of DDT as a plague preventive measure: The Committee listened with great interest to the report of the work being carried out by Dr. M. Sharif at the Haffkine Institute. The experiments described were carried out in jars and artificial rat burrows made to simulate natural burrows. Both DDT and "666" were tested. Both these compounds were found to be very active pulicides, but DDT continued to be active for 140 days and "666" for 57 days. It is proposed to extend this work to natural burrow and link it with field work.

The Committee noted with pleasure that the Director of Public Health, Madras, also proposed to conduct field trials with DDT. To plan and co-ordinate this field work the Committee was of opinion that the Plague Advisory Committee be strengthened by the nomination of some field workers. With this object in view the Committee recommends that the following workers be nominated to the Committee: Dr. P. M. Wagle, Dr. M. Sharif and Dr. P. V. George.

The Committee also recommend that Lieut.-Colonel Sokhey be requested to draw up a comprehensive note on plague work done in the country during recent times to help the strengthened Committee to better plan research work for the future.

MINUTES OF THE FIRST MEETING OF THE CLINICAL RESEARCH ADVISORY COMMITTEE HELD IN NEW DELHI ON THE 24th AUGUST 1945.

Members present :—

1. Lieut.-General J. B. Hance, C.I.E., O.B.E., K.H.S., I.M.S., Director General, Indian Medical Service. (*Chairman*).
2. Lieut.-Colonel A. T. Andreasen, I.M.S., Officer-in-Charge, Surgical Division, Indian Military Hospital, Rawalpindi.
3. Dr. M. N. De, M.B., M.R.C.P. (Lond.), F.N.I., F.S.M.F., Professor of Medicine, Medical College, Calcutta.
4. Lieut.-Colonel H. K. Goadby, R.A.M.C., Assistant Director, Clinical Research, Medical Directorate, General Headquarters, Delhi.
5. Dr. A. N. Goyle, M.D., Ph.D., Professor of Pathology, King Edward Medical College, Lahore.
6. Dr. S. W. Hardikar, M.D., M.R.C.P. (Edin.), Professor of Pharmacology, Osmania University, Hyderabad (Deccan).
7. Rao Bahadur Captain M. G. Kini, M.C., M.B., M.Ch. (Orth.), F.R.C.S.E., F.R.S.E., Superintendent and Professor of Surgery, Stanley Medical College, Madras.
8. Dr. P. Kutumbiah, V.H.C.S., B.A., M.D., M.R.C.P., Principal, Stanley Medical College, Madras.
9. Major Jaswant Singh, I.M.S., Deputy Director, Malaria Institute of India, Delhi.
10. Dr. V. R. Khanolkar, B.Sc. (Lond.), M.D. (Lond.), Director of Laboratories, Tata Memorial Hospital, Bombay. (*Secretary*).

In attendance :—

1. Lieut.-Colonel C. A. Bozman, I.M.S., Public Health Commissioner with the Government of India and Secretary, Indian Research Fund Association.
2. Major T. C. Puri, I.M.S., Deputy Public Health Commissioner with the Government of India.
3. Major C. Mani, I.M.S., Additional Deputy Public Health Commissioner with the Government of India.

Absent :—

1. Sir Lakshmanaswami Mudaliar, Vice-Chancellor, Madras University, Madras.
2. Major F. Sheppard, I.M.S., Professor of Surgery, Superintendent & Surgeon of Surgery, Government General Hospital, Madras.
3. Captain E. J. Somerset, I.M.S., Professor of Ophthalmology, Medical College, Calcutta.

On opening the proceedings, the Chairman of the Scientific Advisory Board, General Hance, apologised for the absence of the Committee's Chairman, Sir Lakshmanaswami Mudaliar, who had been prevented from coming on account of illness. General Hance explained that he had agreed to be chairman at very short notice and that, therefore, had not had the time to study the material submitted by the members as fully as he would have liked. He

had, however, studied a precis of all the notes and before asking individual members to speak on their notes he would like to make a few observations of his own based on what he had gathered from the precis. It seemed that all were agreed that while there was ample material available for clinical research so far little use had been made of this. The Committee might, therefore, be considered as starting from scratch and might well consider first the essentials of clinical research. These he would define as the discriminating use of the five senses, an objective approach to each problem and the development and use of the critical faculties. The Committee would also have to consider how best clinical research could be fostered and young workers be selected, trained and encouraged. He noticed that several members had referred to the position of clinical workers in the teaching colleges and had suggested that those who were to take up clinical research should do so on a whole time basis and should not engage in the active practice of the art. He had discussed this with no less an authority than Professor John Ryle who, he understood, held the view that clinical research and clinical practice and teaching should go hand in hand. He himself certainly endorsed this view. He then requested the members to state their views on the lines suggested by Colonel Bozman in his letter of 12th May, 1945 and asked Dr. Khanolkar to start with his remarks.

Dr. Khanolkar.—Said that as this was the first meeting of the Committee, he regretted the unavoidable absence of Sir Lakshmanaswami Mudaliar, Major Sheppard and Capt. Somerset. Their contributions would have been very helpful to their colleagues. A persual of the notes submitted by the different members of the Committee had shown that they were unanimous on two points. Firstly, that there was little or no clinical research in a big country like India and secondly, that because of the large amount of clinical material available for such research, it was imperative that very early and definite measures should be adopted for its initiation and growth. He said that during his many years of association with the I.R.F.A. he was struck with the fact that, whereas elaborate proposals for work on public health problems and laboratory research were submitted year after year, the proposals for clinical investigations bore the imprint of haste and amateurishness. This had naturally led to two unfortunate consequences. The Scientific Advisory Board with the best of intentions were unable to recommend most of the vaguely conceived proposals submitted to them, and the clinicians were led into an erroneous belief that the I.R.F.A. were not interested in their problems. He should, therefore, like to clear at the outset the latter impression which had no foundation in fact. He had seen that the committees of the I.R.F.A. never rejected on financial grounds alone, any schemes for research which showed even a vague possibility of successful execution. It appeared, therefore, to him that the misunderstanding was due to a faulty design of the problems suggested by clinical workers; and that one of the first cares of this Committee should be to impress on their colleagues in suitable centres, the necessity of putting forward schemes which were clear and complete in their exposition. Unfortunately, a clear presentation implied a clear mind, and a certain amount of experience in asking oneself pertinent questions. It was hoped that this Committee of experienced people would be able to assist their younger associates, by offering them helpful suggestions and timely criticism. He said that all of them realised the great difficulties which beset any attempt at clinical investigation in India, unattended by trained assistance and material facilities. But, he submitted that the first and the main requisite for clinical research, as for any other research, was an urge to explore problems, and that afterwards the other things in the way of

staff and equipment get added on. The Chariman had pointed out that material considerations might have delayed but had never deterred Addison and Bright, and recently Mackenzie and Lewis from undertaking clinical research. Clinical research as had been pointed out was a matter of a reasoned use of five senses, not only in the clinical wards but also in the laboratories. He, therefore, suggested that it was not necessary to wait for elaborate equipment and expert assistance before launching out on a programme of work. A beginning should be made then and there and it was hoped that as the Committee and the medical men working with them gathered experience, the scope of clinical research would be widened and the necessary assistance supplied by the I.R.F.A.

He suggested that the common diseases occurring in different parts of India should be investigated simultaneously at different centres. It would then be possible to correlate the results of several workers and arrive at worthwhile conclusions more expeditiously than by a desultory and spasmodic study which had characterised most efforts in this direction, so far. This Committee was in a favourable position, because it had to build without any past commitments; and though the Committee was still in an embryonic stage, it had the example of other sister committees to encourage it. The Nutrition Advisory Committee, for instance, had grown to be an important body and accquired international reputation in the course of hardly ten years. He said he could remember its humble beginnings and its remarkable growth, which could only be ascribed to the team spirit which actuated most of its members. He then referred to the work which the Committee should undertake at once. He believed that they should first decide on the broad lines on which they should conduct their business at this and at subsequent meetings; and then take up the question of clinical problems which deserved investigation. It may be anticipated, he thought, that properly planned research when it was once set going, would need frequent review and appraisal, and a committee of this type would be best qualified to undertake such work. As the Committee gathered experience and strength, it would be in a position to offer valuable and constructive suggestions to its workers and guide research along productive channels. He hoped to see a large degree of coordination between the different departments in medical institutions, where research will be carried out as a result of the recommendations and activities of this Committee.

Dr. Kutumbiah.—Described some of the considerable local difficulties he had experienced in his own work. He deprecated the lack of interest in research shown by local governments and also the lack of cooperation between different departments in medical colleges. Under existing circumstances it was almost impossible for anyone to initiate any research work and he felt an immediate necessity was an expansion of staff. He favoured the setting up in the teaching institutions of special research units which would be properly i.e. adequately staffed to carry out work and should also be properly though not necessarily luxuriously accommodated. The kind of extra appointments he had in mind were those of technicians, biochemists etc., etc. He left that these were all matters in which they should receive the support of the local government concerned. He felt that the proper housing and indexing of case records called for at least the nucleus of a statistical staff at the large teaching hospitals which he also thought a duty of the local government to provide. He suggested that if all these points were made by the I.R.F.A. who could also point out that the remedy did not really involve heavy financial commitments that such a comment would carry weight with the various governments concerned. He

commented on the practice of the I.R.F.A. giving young men scholarships for one year perhaps extended to two and said that one result of this was that just as soon as a man had learnt the elements of the technique of research he disappeared from the place, the obvious lesson was the necessity for continuity of service whereby young men could be absorbed either by the I.R.F.A. or into larger schemes of work. Without this he doubted if much progress would be made.

Dr. Kini.—Declared he was in general agreement with what the former speaker had said. He thought that research units were necessary on account of the poor departmental facilities now available. He deplored the almost complete lack of departmental cooperation in colleges and of inter-college co-operation. He commented strongly on the grave lack of adequate hospital records and pointed out that it was impossible for one professor to take charge of 35 students and at the same time supervise the maintenance of case records. For this he thought extra staff was absolutely necessary and suggested that post-graduate students might be employed. After all in the absence of any accurate clinical notes no clinical research was possible.

Dr. Goyle.—He agreed with most of what had been said by Dr. Khanolkar. He felt that there was no question but that inter-departmental cooperation was lacking but he had always found that the clinicians were partly responsible anyhow, as they did not themselves follow up their cases. He did not think that the question of permanent appointments was one for the I.R.F.A., he had understood that it was being dealt with by the Health Survey and Development Committee which would doubtless make its proposals in due course. He pointed out that among the young men who got research scholarships under the present system the best of them did get absorbed by the Association or by the Medical Research Department. He agreed with Dr. Kini that better kept records were a *sine qua non*. He thought that no student should be recommended for a scholarship unless he had already worked with the professor recommending him and had shown that he could reach a certain standard. He wished to point out that the promotion of medical research was the concern, not only of the Central Government or bodies like the I.R.F.A. but also of local governments and other public organisations. He was glad to inform the Committee that recently the Punjab Government had placed a sum of Rs. 22,000 at the disposal of the Principal, King Edward Medical College, Lahore, for research, and he hoped that other Governments will follow their excellent example.

Major Jaswant Singh.—Pointed out that all malaria work during the last 15-20 years had been done under the auspices of the I.R.F.A. The functions of the Malaria Institute were well known and the lines of their work were clear from their annual reports. Such included clinical research dealing especially with the problems of treatment. This activity of the Institute had, however, been curtailed on account of the shortage of personnel due to the exigencies of war. No one thought that the enquiries which had been conducted so far on clinical research in malaria represented finality and there could be no difference of opinion on the question of continuing that research. The problem had been considered at the Malaria Institute and it was thought that an immediate and preliminary step was to set up a mobile unit or units to work in some of the hyperendemic, endemic or epidemic areas of the country. It was felt that mobile units would prove more advantageous than permanently located hospitals, inasmuch as the expense would be less and it would be possible to

study the same problems in many different regions. He also stressed the necessity for continuity of the staff engaged in malaria research work.

Professor Hardikar.—Thought that the problem had really three sides namely medical education, medical relief and medical research all of which were closely connected. The personnel available for the first of these i.e. medical education, was far too small, and therefore both medical relief and medical research suffered. Obviously under these conditions the first requirement was more medical workers. On the question of full time research appointments he drew attention to the possibility of research workers acting as consultants in which case part of any fee they got would go to the hospital or institution at which they worked. The success of any such arrangement much depend a great deal on the individual's honesty and academic sense and outlook. He pointed out that one danger of full time well paid appointments was that the wrong man might get appointed. He reminded members that even if the I.R.F.A. had no ultimate authority it carried great weight and he thought that recommendations should be brought specifically to the notice of Governments and College authorities. He strongly recommended that the I.R.F.A. should set up an inspection machinery and pointed out that periodic reports had little value and were a nuisance. He favoured the giving of grants to individuals working on approved plans which should be subject to periodic criticism on inspection. He wanted to see the conditions of work for research workers much improved, at present there were too many difficulties with regard to the position of this class of workers in medical cadres. Finally he thought that men who had been granted research scholarships should be allowed to submit their work as a thesis for post-graduate University degree if considered to be of sufficient merit.

Lt.-Colonel Goadby.—The first point to be settled is the definition of "clinical research". He suggested that clinical research implies dealing with a clinical problem by all methods applicable to it. These consist of accurate observation, accurate record and "sealing wax and string" by which latter he meant the use of all such extraneous aid as might be available or could be devised. One of the essential conditions for entertaining any clinical research was to have proposals submitted to the sanctioning authority in detail. The Clinical Research Advisory Committee should decide in each case whether

(a) the problem had been isolated;
(b) the man concerned was capable of dealing with the problem;
(c) that the problem was capable of solution with the facilities available to the worker at the particular time.

He agreed with the suggestion that work on similar or identical problems may be encouraged at several centres because this increased the likelihood of its yielding quicker answers and supplying greater data for further analysis. It appeared to him that the difficulty in India was that there were few technicians and they were poorly trained. It was, therefore, necessary especially if the funds of the I.R.F.A. were limited, that problems should be selected on which there could be a concentration of energy and available resources rather than diffusing the activities of the clinical research effort. He pointed out certain difficulties which should be clearly borne in mind. It appeared to him that research as such had not yet become attractive in this country and there was often a tendency to direct research from above which in the very nature of things is not possible. It was, therefore, one of the tasks of this Committee to see that clinical research would be made to fit into a pattern and that as a result of coordination and cooperation between different Committees and Institutions that medical research was gradually encouraged in the country.

He remarked that it should be remembered that research workers are born and not created by a stroke of the pen or any other administrative machinery. He hoped that the Medical Research Committee would gradually evolve into a body as important in the development of medical research in India as the Medical Research Council in the United Kingdom. He was very strongly of opinion that the Clinical Research Advisory Committee should come into closer contact with similar and more experienced organisations abroad such as the Medical Research Council, the Rockefeller Foundation, the Carnegie Institute and the Beit Memorial Fund. The exact machinery by which this could be accomplished is a matter which could be decided upon when the Clinical Research Advisory Committee gathers strength. He believed that it may be desirable to get this Committee to meet frequently at short notice as problems which might arise in the course of the year might need immediate solution and it may not be possible to wait for the end of the year to investigate any clinical problem that may be suddenly available. He also very strongly recommended the inspection of all researches financed by the I.R.F.A. and said that there should not be regular inspections but surprise ones at odd times.

Lt.-Colonel Andreasen.—Agreed with Col. Goadby entirely that the main function was to establish a broad policy in which we can build in the future and to this end he said, we should have two sets of programme, (i) with a long view, and (ii) with a short view; and that in the long view a support of the I.R.F.A. would have considerable influence on local Government's support to their local workers. He pointed out that great emphasis should be laid in the choice of problems which was one for the worker and not for this Committee although general direction might be part of the Committee's policy. The question raised by several speakers of cooperation between Departments had been covered by Col. Goadby's remarks. The view that problems should be put up in the greatest detail to the Committee for scrutiny meant of course that all cooperating Departments should have been consulted and their opinions had been taken into consideration before submitting a scheme. The difficulty of non-cooperation would then be got over. He did not think that full-time research posts could be instituted at once; it was necessary to take the long view and aim at the eventual establishment of clinical research under a professor in all colleges in India. He thought that although they should work their way up slowly, that did not prevent them from keeping the future objective in view. He was of opinion that the only criterion about the recognition of quality of research workers' work whether he be a research student or a more experienced worker should be the recognition of its quality by workers outside his own school. He was not against duplication of work because he was sure that it opens up new paths of investigation, either confirmatory or the reverse of the other man's work. He hoped that members of the Committee would not lose their interest in the proceedings after they left this meeting, but would keep in close correspondence during the interval and try and meet quite often. He said that some plan to cover general direction in the next five years would help the Committee to accumulate experience in the direction of research and would probably lead to less waste of effort than merely accumulating individual problems. It would also help to coordinate other workers' efforts. He said that one of the most important functions of the Committee was to establish foreign contacts.

Dr. De.—Was very glad of the opportunity afforded to him by the Committee of explaining some of the difficulties that he had himself experienced. He felt that in his own province research work was practically dead. This h

ascribed to the conditions under which clinical teachers were appointed and had to live. Unless these conditions were materially altered and improved he had little hope for the future. He favoured the idea of research on similar problems carried out simultaneously at different centres but thought that such should not be too near each other as that would lead to unnecessary duplication. He was also strongly in favour of the proposal for periodic inspections as such could be of the greatest value to the worker in guiding them along the right lines. Such inspection would, of course, have to be carried out in the right spirit. He agreed that while it might have no specific authority the I.R.F.A. undoubtedly carried great weight and there was no question but that selection as a research worker under that Association gave an individual a cachet in his local world. It was, therefore, necessary to see that selections were always made with great care.

Lt.-Colonel Bozman.—Described briefly the position of the Committee in relation to its parent body, the I.R.F.A., and said that he felt that the discussions had revealed that there were two sides to the problem under consideration. One might be called the administrative side i.e. the many problems of the pay and position of the research worker, these though of great importance were he suggested scarcely for the consideration of a Clinical Research Committee though he felt that anything they might care to record would be carefully considered and acted on by the Scientific Advisory Board and the Governing Body. The other side of the question was that of proposals for actual research work. Members had themselves advanced a method of approach to this and he suggested that the Committee might now care to consider the kind of problem that should be tackled, the location of work and the personnel that should be employed. He drew attention to the idea that the Committee should have both short term and long term proposals and thought that they would do well to act on that.

General Hance.—Suggested that they might now take as an agenda for discussion the three points made by the last speaker. They should also remember that other Advisory Committees e.g. the Nutrition, Malaria and Cholera Committees all had ideas for incorporation on the clinical side.

Dr. Khanolkar.—Agreed with the chairman's suggested agenda, though he pointed out that any enunciation of problems would relate only to broad fields of investigation.

After discussion at which the members outlined subjects which they considered should be taken into consideration, it was decided that those which appear below should be discussed by members with their colleagues when they go back and should form the basis for specific proposals to be submitted to the Committee at their meeting in November.

1. Peptic ulcer (Madras-Bengal).
2. Infections of the feet (Madras).
3. Rheumatic infections.
4. Problem of essential hyper-tension in India.
5. Cancer in India ; its incidence, clinical varieties and relation to conditions peculiar to the country.
6. Nutrition of surgical cases with special reference to water and mineral balance in Indian patients.
7. Cirrhosis of liver in infants and adults in India.
8. Periosteitis and general nutrition.
9. Investigation into the nutrition conditions of anaemias of poorer classes.

10. A study of relapses and reinfection problems of Malaria in different areas of India.
11. Amoebiasis.
12. The complications of arsenical treatment.
13. A study of poliomyelitis in its earlier stages.

The list was not arranged either in order of importance or urgency; nor was it suggested that it exhausted the possibilities of fields of clinical investigation in India.

The Committee then proceeded to pass the following resolutions:

(1) The Clinical Research Committee recommend that every effort should be made to institute and co-ordinate clinical research in India under the auspices of the Indian Research Fund Association.

(2) The Committee recommend an early establishment of close contact between this Committee and recognised research organisations such as Medical Research Council, Rockefeller Foundation, etc.

(3) The Committee recommend that their annual meeting should take place in Delhi along with that of the other Advisory Committees. In addition to the annual meeting they should hold at least one other meeting which should assemble at a centre at which clinical research is being carried out at least five months before the annual meeting.

(4) This Committee are of opinion that the method of keeping clinical records in medical institutions is unsatisfactory and that steps should be taken by the authorities concerned to remedy this defect as they are of the opinion that without such records no clinical research can be undertaken, nor a proper basic training of medical students carried out.

(5) The Committee at this meeting have suggested several general fields of work within which specific problems might be chosen by individuals. This in no way limits the work to these fields.

(6) The Clinical Research Advisory Committee should select from amongst proposals submitted to them annually such as they consider capable of solution by the personnel concerned and the facility that can be made available to them.

(7) The Committee suggest that proposals for the programme of work must be submitted in fullest detail and should include a review of the present knowledge in the proposed subject.

(8) This Committee are of opinion that all clinical research work in progress will be open to inspection by persons recommended by this Committee for that purpose.

Dr. Khanolkar expressed the gratitude of the Committee to General Hance, who had consented to preside at its inaugural meeting at such short notice, and had launched it on its uncharted career with much sympathy and skilful guidance. He wanted the Committee to know that it owed its existence to the interest in clinical research evinced by General Hance, since taking up office as Director General. He was sure that he was expressing the feelings of all the members present, when he said that he hoped that General Hance would continue to take the same interest in the affairs of this Committee during the difficult period of its infancy, as he had in its origin.

General Hance then thanked those present for the valuable contributions made by them during the day's deliberations, and he hoped that as a result of their labour they had laid the foundations of an important undertaking in the future development of the country.

Report of the Second Meeting of the Clinical Research Advisory Committee held in New Delhi on the 29th November 1945.

Members present:—

1. Lieut.-General J. B. Hance, C.I.E., O.B.E., K.H.S., I.M.S., Director General, Indian Medical Service. (*Chairman*).
2. Brigadier H. K. Goadby, R.A.M.C., Consulting Physician, Southern and Eastern Commands, Bangalore.
3. Dr. A. N. Goyle, M.D., Ph.D., Professor of Pathology, King Edward Medical College, Lahore.
4. Rao Bahadur Captain M. G. Kini, M.C., M.B., M.Ch. (Orth.), F.R.C.S.E., F.R.S.E., Superintendent, Stanley Hospital, Madras.
5. Dr. P. Kutumbiah, V.H.C.S., B.A., M.D., M.R.C.P., Principal, Stanley Medical College, Madras.
6. Major F. Sheppard, I.M.S., Superintendent and Surgeon of Surgery, Government General Hospital, Madras.
7. Major Jaswant Singh, I.M.S., Deputy Director, Malaria Institute of India, Delhi.
8. Captain E. J. Somerset, I.M.S., Professor of Ophthalmology, Medical College, Calcutta.
9. Dr. V. R. Khanolkar, B.Sc. (Lond.), M.D. (Lond.), Director of Laboratories, Tata Memorial Hospital, Bombay. (*Secretary*).

In attendance :—

1. Lieut.-Colonel C. A. Bozman, I.M.S., Public Health Commissioner with the Government of India & Secretary, Indian Research Fund Association.
2. Sir Shanti Sarup Bhatnagar, Director, Council of Scientific & Industrial Research, New Delhi.
3. Lieut.-Colonel C. L. Pasricha, I.M.S., Director, School of Tropical Medicine, Calcutta.
4. Lieut.-Colonel C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene & Public Health, Calcutta.
5. Dr. C. G. Pandit, O.B.E., M.B.B.S., Ph.D., Director, King Institute, Guindy, Madras.
6. Dr. S. R. Pandit, M.B.B.S., B.Sc., D.E., Director, Pasteur Institute, Shillong.
7. Dr. Radhakrishna Rao, Assistant Director, Department of Nutrition & Experimental Pathology, Haffkine Institute, Bombay.
8. Mr. T. Gonsalves, B.A.(Hons.), LL.B., Assistant Secretary, Indian Research Fund Association.
9. Dr. (Miss) Kamala Bhagvat, M.Sc., Ph.D. (Cantab.) Assistant Director, Nutrition Research Laboratories, Coonoor.
10. Dr. S. Pandit, W.M.S., Director, Maternity & Child Welfare Bureau, Indian Red Cross Society, New Delhi.

In the absence of Sir Lakshmanaswami Mudaliar who was unfortunately prevented on account of illness from attending the meeting Lieut.-General Hance, Chairman of the Scientific Advisory Board, took the chair. Apologies for absence were also received from Professor Hardikar, Dr. M. N. De and Lieut.-Colonel Andreasen.

I. The minutes of the first meeting of the Clinical Research Advisory Committee held in New Delhi on the 24th August, 1945, were adopted.

II (a). The Committee considered para. 6 of the report of the Cholera Advisory Committee which was held in Simla on the 9th June 1945 and welcomed the liaison proposed by that Committee.

(b). The Committee considered para. 8 of the minutes of the Industrial Health Advisory Committee which met in New Delhi on the 5th November 1945 and welcomed the liaison suggested by that Committee. They also suggested that that Committee might with profit be strengthened by the addition of two clinicians. They suggested that in this connection Dr. Kini and Dr. De might be invited.

III (a). The Committee considered the attached note entitled "A study of water and salt requirements in Indians" by Dr. V. R. Khanolkar, Director of Laboratories, Tata Memorial Hospital, Bombay. After discussion Dr. Khanolkar pointed out that his note had been chiefly intended to stimulate interest in the problems referred to. It was unanimously agreed that work on these lines should be prosecuted.

(b) The Secretary of the Association drew the attention of the Committee to the discussion which took place at the Nutrition Advisory Committee on the subject of lathyrism and its associated problems. The Committee noted that the Nutrition Advisory Committee had recommended that Dr. B. M. Roy, Nutrition Officer, Central Provinces, should consult with research workers who are at present carrying out work on lathyrism with a view to drawing up a plan of work.

IV. The Committee reviewed the following enquiries and recommended their continuation:

(i) Clinical Research Unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

(ii) Enquiry on Naga sore under Dr. S. R. Pandit, Director, Pasteur Institute, Shillong.

(iii) Enquiry into hypoproteinaemia and other conditions associated with malnutrition under Dr. John Lowe at the School of Tropical Medicine, Calcutta. This enquiry was recommended for one year more.

(iv) Enquiry into erythroblastosis foetalis and its association with Rh factor under Lieut.-Colonel S. D. S. Greval, I.M.S., Imperial Serologist to the Government of India at the School of Tropical Medicine, Calcutta.

This enquiry was recommended for one year only. The Committee also recommended that when the Scientific Advisory Board are considering priorities for enquiries they should consider what priority should be allotted to this enquiry.

(v) Enquiry on medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

The Committee considered that investigations into mycology should continue but that the proposals submitted were not acceptable and that the authorities responsible should be asked to submit a more definite line of research.

The Committee noted that work on the enquiry on hill diarrhoea under Dr. G. Panja at the School of Tropical Medicine, Calcutta, had been completed.

PROPOSALS FOR NEW ENQUIRIES.

(i) Enquiry on nitrogen metabolism during the post operative period in surgical cases under Dr. Syed Ahmad and Dr. U. P. Sinha at the Public Health Laboratories, Patna.

Not recommended.

(ii) Enquiry on bone marrow under Dr. D. Govinda Reddy, Professor of Pathology, Medical College, Madras.

Not recommended.

(iii) Enquiry into infections of the feet under R.B. Dr. M. G. Kini, Superintendent, Stanley Medical College Hospital, Madras.

The Committee recommended this enquiry on the understanding that work will be confined to items 1 and 2 of the note submitted by the sponsor of the proposal.

(iv) Enquiry on bacteriophage theraphy in typhoid fever under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

Not recommended.

(v) Enquiry on the effects of penicillin in diphtheria under Dr. M. N. De, Professor of Medicine, Medical College Hospital, Calcutta.

Not recommended.

(vi) Enquiry on cirrhosis in animals and its bearing on the aetiology of infantile biliary cirrhosis under Dr. T. Bhaskara Menon, Professor of Pathology, Andhra Medical College, Vizagapatam.

(vii) Enquiry on infantile cirrhosis of the liver under Prof. V. Subrahmanyan at the Indian Institute of Science, Bangalore.

(viii) Enquiry on infantile cirrhosis under Dr. P. Kutumbiah at the Stanley Medical College, Madras.

The Committee considered these three together as they relate to similar problems. Numbers (vi) and (viii) were recommended and number (vii) was not recommended.

In making their recommendation the Committee noted that the Nutrition Advisory Committee had already considered an enquiry by Dr. Radhakrishna Rao, Bombay, on this problem and in order that work should not overlap they strongly recommended that the three workers concerned, viz., Prof. Kutumbiah, Prof. Menon and Dr. Radhakrishna Rao should meet and on consultation with Dr. Damodaran draw up a concerted plan of work for next year before undertaking individual investigations.

(ix) Enquiry into the etiology and pathogenesis and clinical manifestations of splenomegaly of unknown origin under Dr. M. N. De, Professor of Medicine, Medical College, Calcutta, and Dr. B. P. Tribedi, Professor of Pathology, Medical College, Calcutta.

Not recommended.

(x) Enquiry into venereal diseases in Calcutta under Lieut.-Col. S. D. S. Greval, Imperial Serologist to the Government of India at the School of Tropical Medicine, Calcutta.

Not recommended.

(xi) Dental Research Unit under Dr. C. D. Marshall Day, Principal, DeMontmorency College, of Dentistry, Lahore.

Recommended. The Committee recommended that the sponsor of this enquiry should be asked whether with regard to item (b) of his proposals he had the necessary facilities available. In this connection Dr. Goyle stated he might be able to afford the necessary laboratory facilities needed for this aspect of the work.

(xii) Enquiry on the relationship between the measurements of vertebrae and body size of Indians under Dr. R. G. Ginde at the Seth G. S. Medical College, Bombay.

The Committee in recommending this enquiry pointed out that before work be started the individual sponsoring the enquiry should be asked to supply further details on consideration of which the necessary allotment of funds will be made.

(xiii) Enquiry on the incidence and varieties of tumours occurring in the tropics under R.B. Captain K. S. Nigam, Head of the Department of Surgery, King George's Medical College, Lucknow.

Not recommended.

(xiv) Enquiry into causes of flat feet in Indians under the Head of the Department of Physiology, King George's Medical College, Lucknow.

Not recommended.

(xv) Enquiry into carbohydrate metabolism with special reference to the cause and theory of diabetes under Dr. M. C. Nath at Dacca University, Dacca.

The Committee noted that the problem to be investigated had not been clearly stated. They considered that the worker concerned should be asked to isolate in better detail the problem that he wishes to investigate and that if this is satisfactory sanction might be accorded.

(xvi) Enquiry on closed head injuries under Lieut.-Colonel B. S. Nat, I.M.S., at the Irwin Hospital, Delhi.

This was withdrawn by the sponsor.

(xvii) Enquiry into causes of blindness under Captain E. J. Somerset, I.M.S., Professor of Ophthalmology, Medical College, Calcutta.

Recommended.

(xviii) Enquiry on beri beri, pellagra, riboflavin deficiency, post puerperal neuritis under Dr. T. K. Raman, Professor of Therapeutics, Andhra Medical College, Vizagapatam.

Not recommended.

V. This Committee recommends to the Scientific Advisory Board that when calling for proposals for new enquiries a directive in the following terms should invariably be issued along with the information afforded to persons concerning proposals to be placed before the Advisory Committees. The following criteria must be borne in mind by research workers in order that those who are responsible for considering the giving of grants shall be able to assess accurately the value of the work to be done.

(1) The exact problem to be investigated should be isolated and the question before the investigator clearly defined and stated. Each research

proposal should be concerned with one specific problem and should not entail the investigation of a number of other problems.

(2) The lines of work should be given in detail and the assistance that may be available from associated workers e.g. biochemists, radiologists etc. should be mentioned with the name of such associates.

(3) The worker should state his previous experience of working on this or allied problems.

(4) A brief resume of all the relevant literature should be given. This is not only to ensure that needless repetitions are avoided but that the worker is aware of all previous work that has been done. The bare statement that the work contemplated has not been carried out in this country previously is no necessary recommendation that it should be carried out now.

(5) It is very important in the planning of biological investigations and surveys involving statistical principles specially regarding such problems as therapeutic trials to ensure that time and effort should not be wasted. Therefore the worker is advised to consult a competent statistician at his own centre in the planning of his work and to indicate that this has been done when submitting his proposal.

(6) It is important that a problem should be limited enough to be soluble within a reasonable time. It may be pointed out that several problems of identical or similar nature may be before the Indian Research Fund Association at the time and the Association may suggest a slightly different approach to the research workers to bring all investigations into line.

Note on a programme of work for a study of water and salt requirements in Indians by Dr. V. R. Khanolkar, Director of Laboratories, Tata Memorial Hospital, Bombay.

The water and salt requirements of human beings have occupied the attention of many investigators, because it was realised that almost seven-tenths of the body is made up of water and that all its principal biological reactions take place in a saline fluid medium, to which other constituents may have been added. Nearly 100 years ago Claude Bernard (1) elaborated his conception of a liquid " milieu interieur " which surrounds and bathes the cells. Later work has demonstrated the correctness of this conception and recent work has shown the significance of the stability of composition of this fluid medium. Its role in the evolution of living organisms from an environment similar to the sea water, to an environment prevailing on dry land has begun to be fully appreciated. " The history of the extra-cellular fluid is clearly indicated by the resemblance of its chemical pattern to that of the sea water ".

The fluid components of the body are the intra-cellular fluid (50% B.W.), the interstitial fluid (15% B.W.) and the blood plasma (5% B.W.). The water moves between intra-cellular and extra-cellular spaces in such a manner that it tends to preserve an ostomic equilibrium between the fluid inside the cells and that in which they are immersed. About 90% of the osmotic pressure of body fluids is exercised by the electrolytes which they contain ; and the distribution of water between the three components is mainly determined by the migration of electrolytes. The interstitial fluid is the only fluid which is capable of variations during physiological processes e.g. digestion, sleep, profuse sweating, or pathological reactions such as fever, shock, diarrhoeas etc. This fluid acts as a buffer during health and disease, with a view to maintaining a constancy in the character of the intra-cellular fluid and the blood plasma. Careful studies of many pioneer workers (Dill, Gamble, Henderson, Peters and VanSlyke) and recently of Smith, Darrow, Widowson and McCance have shown that the human body has evolved very delicate mechanisms for maintaining the stability of the fluid medium, and that all conditions which alter even to a small extent for any length of time the composition and content of the fluids, interferes markedly with the normal functions of the body.

Most of our knowledge of the water and salt requirements of the body are based on data derived from observations in temperate climates. The conditions in the tropics, however, with higher temperatures and sometimes a more humid atmosphere considerably alter the normal requirements of the body. This was particularly brought home during the last six years when men accustomed to a temperate environment throughout the major part of the year were required to carry out strenuous exertion, associated with modern combat conditions in tropical areas. Investigations were therefore undertaken on persons undergoing military training in American desert area (—), and on subjects spending several days on pneumatic rafts in the Gulf of Mexico (—).

Heat is being continually generated within the body as a result of the chemical processes associated with metabolism. This heat is dissipated from the skin to the environment. In warm climates the secretion of sweat and its evaporation, is the main factor in the dissipation of heat from the skin. The rate of evaporation is greater, the higher the wind speed, and the lower the humidity of the air.

The volume of sweat lost during eight hours of strenuous work has been found to be as high as 10-15 litres. Brunt (2) has plotted out curves showing the limits of range of tolerable comfort, in human subjects for varying conditions of temperature and humidity. A nude subject resting in shade at a temperature of 110°F., a relative humidity of 42%, and a wind velocity of 11 miles per hour would lose water by sweating at a rate of at least 2 pints (1134 c.c.) per hour. The loss of water and the consequent dehydration may play an important part in the reactions of people living in hot humid climates during health and disease. The importance of loss of water by sweating is much greater because sweat is not pure water but contains besides organic and inorganic substances, about 3-4 gms. per litre of Sodium Chloride. The salt which is thus lost to the body by sweating would lead to a gradual development of salt deficiency if it is not replaced in time. Such deficiency is associated with certain well known subjective and objective symptoms e.g. weakness, early fatigue, anorexia, nausea, and an impairment of physical performance and mental acuity. In India, the conditions are further complicated by a customary use of a vegetarian diet by a large mass of people and a poorer intake of animal proteins. Such diet is poorer in Na and richer in K than the habitual diet of most Europeans and Americans. The effects of these peculiar conditions on the health of normal individuals and on their resistance to infections have not been satisfactorily worked out in India or in any part of the world with similar physical environment, as far as could be ascertained by a perusal of available literature. The general considerations stated above suggest the necessity for a planned investigation of salt and water metabolism in India.

Plan of work.

The work may be divided in two parts:

I. A study of the normal requirements of salt and water in healthy subjects under differing conditions of temperature, humidity, ventilation and exertion. This study would form the basis of all work of this nature on patients and may be undertaken on a uniform plan in different parts of the country.

II. A study of salt and water requirements in clinical conditions.

(a) *Surgical*:—Shock due to trauma, burns and operative procedures. A considerable amount of work has been done in other countries on this problem. The work in India would be more in the nature of a corroborative investigation, under the difficult conditions encountered by surgeons in India, and which may be attributable to temperature, humidity and malnutrition.

(b) *Medical*:—Infantile diarrhoeas and cholera offer ample opportunities for studying this problem. It is suggested that a clinical and laboratory investigation of this type should be undertaken by a team of trained workers who have familiarised themselves with the plan and the techniques mentioned above.

Methods of investigation.

These would mainly consist of an estimation of the interstitial fluid and plasma and an accurate determination of salt and water intake and the output over a given period of time. The following tentative programme of work may be suggested:

1. The concentration of electrolytes in the blood plasma is shown in the following table:

Bases	m Eq, per litre	Acids	m Eq, per litre
Sodium ..	.. 140	Chloride ..	.. 104
Potassium ..	.. 4	Bicarbonate	.. 25
Calcium ..	.. 5	Proteinate	.. 17
Magnesium ..	.. 2	Phosphate ..	.. 2
		Lactate ..	.. 1
		Undetermined	.. 2
Total	.. 151	Total	.. 151

It would be seen that among basic ions the cations Na and among acid ions, the anions chloride and bicarbonate predominate in the extra-cellular fluid. An estimation of the total extra-cellular fluid and the concentration of Na, K, total base, Cl, bicarbonate and protein in serum of normal healthy individuals would constitute a first step in this work.

2. An accurate analysis of the total intake of water and salt and their output during 24 hour periods for studying the balance of these substances in healthy Indians. It is generally acknowledged that human beings can accustom themselves to living and working most efficiently in an environment in which they are born, and have their being. These studies may show that most Indians have adapted themselves more advantageously to their particular environment than people from temperate climates could be expected to do during short periods of residence in the country.

3. It has been noticed that during severe dehydration e.g. in asiatic cholera, there is a significant rise in blood urea (3–4). The relation between the function of the kidneys and the ionic pattern of the blood is an important subject which deserves a deliberate study and it may be suggested that the methods employed for 1 and 2 above will supply a basis for undertaking the investigation outlined under this head.

4. A study of the correlation between acidosis and alkalosis, and the sodium chloride and bicarbonate concentration in blood is likely to shed some light on the clinical condition of acidosis which is so frequently met with in India.

It is hoped that the practical applications of these studies will lead to the adoption of a more reasoned line of treatment in several clinical conditions.

NOTE :— A reasonably complete review (with bibliography) of this subject has recently appeared in Annual Review of Physiology (1944, 1945) Vols. 6 and 7. A list of references is therefore not appended here, except in the case of publications referred to in the text above which have not been listed in these reviews.

REFERENCES.

1. Claude Bernard : Lecons sur les proprietes physiologiques et les alterations pathologiques des liquides de l'organisme : Paris, 1859.

2. Brunt, D. : Climate and Human Comfort. Nature, 155 : 559 : 1945.

3. Pasricha, C. L., & Malik, K. S. : The Blood in Cholera. Ind. Jour. Med. Res. **28** : 301-307 : 1940.

4. Black, D. A. K., McCance, R. A., & Young, W. F. : A Study of Dehydration by means of Balance Experiments, Jour. Physiol. 102 : 406-414 ; 1944,

REPORT OF THE MATERNITY AND CHILD WELFARE ADVISORY COMMITTEE.

The meeting was held at 10-A.M. on November 26th, 1945, in New Delhi.

PRESENT.

Dr. J. Jhirad.	(*In the Chair*).
Lieut.-Colonel H. M. Lazarus, W.M.S.	
Dr. A. Jean M. Orkney, W.M.S.	
Dr. Benjamin.	(*Invitation*).
Lieut.-Colonel C. A. Bozman, I.M.S.	—Do.—
Lt.-Colonel C. K. Lakshmanan, I.M.S.	—Do.—
Major T. C. Puri, I.M.S.	—Do.—
Major C. Mani, I.M.S.	—Do.—
Major B. N. Khan, I.M.S.	—Do.—
W/Cdr. Shrinagesh, M.M.	—Do.—
Dr. S. Pandit, W.M.S.	(*Secretary*).

Apology for absence was received from Dr. M. I. Neal Edwards.

1. The Chairman called upon Colonel Bozman to speak on the note he had sent to the Secretary of the Advisory Committee regarding Maternity and Child Welfare in India, and the formulation of plans for the advancement of research on the subject.

Colonel Bozman explaining the note, stressed the significance of these subjects, putting them next in importance to malaria as fields for research.

He suggested that the functions of the Advisory Committee should include, besides research in the various problems, enquiries into the training of personnel both medical and other public health, field workers in order to improve the machinery for application of the suggestions accruing from various findings in the course of research. This was discussed freely by the members and it was unanimously felt that the training in public health and social medicine to medical students and health workers should be modified so as to give a practical trend and to inculcate a higher sense of responsibility towards and need for research in this particular branch of preventive medicine.

The following resolution was passed unanimously :

" The subject of Maternity and Child Welfare calls for a great increase in research into the many factors that underlie the high mortality and morbidity figures that are associated in India with a normal physiological function and the course of an individual's early years of life. Such research in the absence of facilities for applying the results are barren. Necessary preliminary is, therefore, the immediate improvement of the teaching and presentation of this subject in medical colleges. Concurrently, the training of health visitors and conditions of service of both medical and nursing personnel in the public health field require drastic reforms. Standardization of records would facilitate research.

This Committee considers that steps which could, and should be taken now by the central and provincial Governments are the immediate appointment of advisers in maternal and child health at headquarters and the taking over of all responsibilities in connection with this work."

2. The Committee considered the following resolution sent up by the T.N.A.I. and gave it their full support:

"Members of the Health Visitors League of the T.N.A.I. assembled in Conference in Delhi on 20-11-45, strongly recommended a uniform standard of training in every Health School, as the existing standard of training varies in various Provinces."

3. In view of the importance of research in this branch of preventive medicine the Committee recommends the desirability of having a medical woman on the Scientific Advisory Board.

4. The Committee discussed the desirability of having trained personnel for initiating and guiding research in the various provinces. In this connection it was suggested that the Women's Medical Service may be approached to lend a member of their senior service. If the proposal is acceptable to the Scientific Advisory Board it is estimated that an annual provision of Rs. 8,880/- to cover special allowance in lieu of private practice (Rs. 400/- p.m.); house allowance (Rs. 150/- p.m.); travelling allowance (Rs. 1,000/- p.a.); leave allowance (Rs. 800/- p.a.) and contribution towards Provident Fund (Rs. 80/- p.a.) will be necessary. The Women's Medical Service would, it is felt, meet the regular salary of the officer. It is anticipated that each province putting up schemes for research will require a grant of Rs. 10,000/- to Rs. 15,000/- according to the subject selected.

5. *Existing enquiries.*—(i) Enquiry into infant and child mortality and morbidity in Calcutta, under Dr. J. Orkney, W.M.S., at the All-India Institute of Hygiene and Public Health, Calcutta.

(ii) Enquiry into anaemia in Pregnancy in Singur Health Unit, under Dr. J. Orkney, W.M.S., Professor of Maternity and Child Welfare at the All-India Institute of Hygiene & Public Health, Calcutta.

Dr. Orkney reported that owing to want of suitable staff, work could not be started, but she felt that the enquiry into anaemia in pregnancy in Singur could be undertaken next year. The Committee recommends that the grant already sanctioned be held over for 1946-47.

6. *Completed enquiries.*—Dr. Ghosh's reports on, (a) Infant Mortality, and (b) Standards of Maturity for Indian infants, were placed on the table. The Committee suggests appointment of a Sub-Committe to consider these reports.

7. *New enquiries.*—(i) Enquiry on prenatal and postnatal growth, under Dr. Khan, Professor of Anatomy, Lucknow.

The Committee did not consider this favourably.

(ii) Enquiry into pelvic type and its relationship to body build in Indian Women, under Drs. S. K. Basu, S. Mitra and S. Mukerjee, Calcutta.

The Committee felt that such research has high academic value and is likely to have a bearing on practical problems, but such investigation could be carried out with the help of the machinery and personnel existing in teaching institutions. However, the Committee recommends a grant of Rs. 2,500/- towards cost of X'ray plates.

(iii) Enquiry into the bearing of premature and immature births on infant mortality, under Dr. B. C. Das Gupta, Bombay.

The Committee felt that such an enquiry is of immense importance and recommends it strongly for prior consideration.

8. *Suggestions for future research.*—The Committee felt that the following subjects need early investigation in the order stated :

Clinical Subjects :

(a) Puerperal Sepsis
(b) Study of Pyorrhea
(c) Osteomalacia
(d) Anaemia
(e) Feeding of infants & toddlers
(f) Normal neonatal standards

Statistical subjects :

(a) Survey of infant mortality and morbidity.

(b) Effect of large families and frequent child bearing on infant mortality and morbidity.

9. A note on "Calculation of Infant Mortality Rates from the Records of Maternity and Child Welfare Centres", by C. Chandrasekaran, Professor of Statistics, All-India Institute of Hygiene and Public Health, Calcutta, was considered and it was suggested that copies be sent to Municipalities of Bombay, Delhi, Calcutta, Madras and Bangalore, to the different Health Units as also to the Director of Public Health, Madras, Bengal and Delhi.

10. The Committee was referred to Minutes of 1941 (p. 136 C) where a suggestion was made to publish a popular Bulletin embodying the findings of the maternal mortality enquiries. It was considered that wider publicity to the findings and recommendations was necessary and it was decided to urge the preparation of the popular Bulletin.

RABIES ADVISORY COMMITTEE

The Committee met at 10 a.m. on November 26, 1945. It was constituted as follows :—

Major-General G. Covell (*President*).
Lt.-Col. M. L. Ahuja.
Lt.-Col. H. W. Mulligan.
Dr. S. R. Pandit.
Dr. S. R. Savoor.
Dr. N. Veeraraghavan.
Dr. A. G. Brooks (*Secretary*).

The following were also present :—

Bt.-Col. Sir Ram Nath Chopra.
Dr. B. B. Dikshit.
Dr. V. R. Khanolkar.
Dr. C. G. Pandit.
Lt.-Col. C. L. Pasricha.
Lt.-Col. S. S. Sokhey.

PROCEEDINGS.

1. In his opening address the Chairman said that the Rabies Advisory Committee had been revived for the purpose of stimulating further research on rabies. Members of the Committee had been asked to submit notes embodying their views on the future of rabies research in India and these had been circulated. The Chairman reviewed the various suggestions which had been made and said that it was obvious from these that there were many pressing problems which required attention. In the Chairman's opinion India was capable of providing better facilities for research work on rabies than any other country in the world.

2. The suggestions made for further research on rabies included improved methods for the preparation and standardisation of antirabic vaccine, for the diagnosis of rabies, and for the study of the mode of spread of rabies virus in the body. The urgent necessity for initiation of further research was agreed to unanimously particularly as regards the improvement of the antigenic value of antirabic vaccine and the removal of the risk of paralytic accident.

3. The Committee agreed that if any real progress were to be made in the improvement of the antirabic vaccine at present in common use it would be necessary to embark on a long term research programme to ensure continuity of the work over a period of at least 10 years. The Committee recognised that the primary requirements for this work were (a) the recruitment of suitable workers and (b) the provision of adequate facilities for the work to be undertaken.

The Committee heard with interest the results of the research work on antirabic vaccine recently carried out by Dr. Veeraraghavan at the Pasteur Institute, Coonoor, and recorded their appreciation of his work. The Committee felt that it was essential that Dr. Veeraraghavan's experiments should be repeated, confirmed and extended by independent workers.

4. The Committee considered that the existing facilities for rabies research at the Pasteur Institute, Coonoor, were probably the best available in India at the present time and recommended that these should be strengthened by the

provision of additional staff and equipment. The Committee also recognised the need for a second centre for research work on antirabic vaccine which should provide for the conduct of animal experiments on a large scale but recognised that no adequate facilities existed elsewhere in India for work of this kind. In this connection the Committee unanimously agreed that the attention of the Association of the Pasteur Institute of India should be directed to this need and that the Association be asked to consider the provision of the funds necessary to establish a suitable centre for rabies research. The Director, Central Research Institute, Kasauli, put forward a suggestion that a suitable centre for rabies research should be created at the Institute by the acquisition of an adjacent site, the most suitable being the property known as "Widdicombe" belonging to His Highness the Maharaja of Patiala. The buildings on this site are such that they could be readily converted into suitable work rooms and animal houses.

5. The Committee recommended that workers at other institutes in India which were engaged on rabies work should be encouraged to undertake research work on this subject in so far as facilities permitted. Proposals regarding lines on which work should be carried out should be forwarded to the Secretary, I.R.F.A. for consideration and co-operation by Rabies Advisory Committee.

Minutes of the first meeting of the Industrial Health Advisory Committee held in New Delhi on the 5th November 1945.

MEMBERS PRESENT.

1. Lieut.-Colonel C. A. Bozman, I.M.S., Public Health Commissioner with the Government of India & Secretary, Indian Research Fund Association (*Chairman*).
2. Mr. S. C. Joshi, M.A., LL.B., Chief Labour Commissioner, Government of India, Department of Labour.
3. Major E. L. Jones, I.M.S., Additional Deputy Director General (Social Insurance), Indian Medical Service, New Delhi.
4. Lieut.-Colonel C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene & Public Health, Calcutta.
5. Dr. J. M. Orkney, W.M.S., Professor of Maternity & Child Welfare, All-India Institute of Hygiene & Public Health, Calcutta.
6. Dr. V. N. Patwardhan, M.Sc., Ph.D., Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.
7. Mr. M. T. Raju, I.C.S., Deputy Secretary to the Government of India, Department of Labour, New Delhi.
8. Dr. J. N. Ray, Ph.D., D.Sc., Director of Drugs & Dressing, Office of the Controller of Purchase, Medical Division, Department of Supply, New Delhi.
9. Dr. M. N. Rao, M.B.B.S., Dr. P. H., Assistant Professor in charge of Physiological Hygiene, All-India Institute of Hygiene & Public Health, Calcutta (*Secretary*).

IN ATTENDANCE.

1. Mr. T. Gonsalves, B.A. (Hons.), LL.B., Assistant Secretary, Indian Research Fund Association.
2. Major T. C. Puri, I.M.S., Deputy Public Health Commissioner with the Government of India.

Lieut.-General J. B. Hance, C.I.E., O.B.E., K.H.S., I.M.S., Chairman of the Scientific Advisory Board kindly attended the meeting.

After the Chairman had introduced the various members of the Committee to each other, General Hance, Chairman, Scientific Advisory Board of the I.R.F.A. spoke on the subject of industrial health. He pointed out that unhealthy labour means diminished output and is, therefore, a waste of human effort and energy. He anticipated that the activities of the Committee would disprove previous accusations raised against research programmes of the past of which it was said that they were more connected with laboratory work and with experimental animals than with the actual health conditions of the masses.

2. The Chairman then suggested to the Committee that their work concerned the study of conditions for the maintenance of health and efficiency in industrial workers and they might agree that this should be approached from the following view points :—

(i) the study of diseases peculiar to industry which might be briefly termed occupational diseases;

(ii) the study of conditions especially liable to develop in industrial workers;

(iii) the conditions obtaining in mines, factories, and other industries in regard to hygiene, light, ventilation, safety factors for workers, welfare work, canteens, housing conditions etc;

(iv) the health of women in industry;

(v) the maintenance of health and sickness records.

3. After preliminary discussion on these items the Committee agreed that the starting point for research on industrial health was the maintenance of adequate health and sickness records. It was also agreed that the maintenance of such records called for personnel who had adequate training in industrial health problems. As matters stand at present there is no general and uniform system of maintaining records of absenteeism due to illness or other reasons and this is in part due to the fact that the personnel who maintain industrial records do not have adequate training in regard to matters connected with industrial health. The Committee noted that a start was being made to encourage interest in medical industrial workers in matters concerning industrial health by a course of instruction for physicians at the All-India Institute of Hygiene and Public Health, Calcutta. The Committee considered that this small beginning was a step in the right direction and that efforts should be made to give similar courses of training to certifying Surgeons, Factory Inspectors, Industrial Nurses and Health Visitors. It was pointed out that at present there are in India no public health nurses with experience of industrial health nursing. The Committee felt that a recommendation should be made to the Government of India to arrange for the training of such personnel abroad.

4. The Committee discussed in detail the question regarding the maintenance of records of health and sickness among factory workers. The general trend of opinion was that under present methods and with the present staff employed accurate data which could be used by a research worker was not available. After some discussion it was decided that this question be referred to a Sub-Committee consisting of (1) Lieut.-Colonel C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene and Public Health, Calcutta, (2) Mr. S. C. Joshi, Chief Labour Commissioner with the Government of India, Department of Labour, New Delhi and (3) Dr. M. N. Rao, Assistant Professor of Physiological and Industrial Hygiene, All-India Institute of Hygiene and Public Health, Calcutta. The Sub-Committee was to examine the present position with a view to see whether the introduction of legislation to enforce the maintenance of adequate records is a practical proposal. It was pointed out by one of the members that this question would require careful examination and involved the examination of the Industrial Statistical Act, the Factories Act and other legislation.

5. The fields of research into occupational diseases were discussed at length. It was felt that at this stage the Committee should make recommendations regarding research that should be undertaken in connection with occupational diseases connected with the Textile Industry; Railways, Engineering, Posts and Telegraphs, Mines (Coal, Manganese, Salt, Mica, Gold) and the Tanning Industry. The possibility of welcoming research projects from specific persons and organisations was also considered.

6. During the discussion it was decided that the question of the health of women in industry in India was a subject calling for early attention. In this connection it was pointed out that with effect from 1st February, 1946, women will not be permitted to work in mines underground, and it was felt that a limited enquiry, taking random samples of health conditions of women workers underground, should be started immediately so that some data regarding their health might be collected which could be utilised at a later date for purposes of comparison with women workers who are employed on the surface. The Committee recommended that this enquiry should be conducted under the direction of Dr. Jean Orkney, Professor of Maternity and Child Welfare, All-India Institute of Hygiene and Public Health, Calcutta, and that she should work out the details of the staff she would require and submit a note on the subject to the Secretary, Governing Body & Scientific Advisory Board, Indian Research Fund Association, immediately.

7. The Chairman suggested to the Committee that the question of training industrial health personnel and the formulation of detailed proposals for research in industrial health might await the recommendations of the Health Survey & Development Committee which had considered both subjects at length.

8. The Chairman informed the Committee that the Scientific Advisory Board of the I.R.F.A. was advised with regard to clinical research by its Clinical Research Advisory Committee. On the advice of the Chairman the Committee recorded that in matters concerning industrial research, liaison should be maintained with that Committee.

9. The Committee discussed generally the question of the welfare of workers. They felt that while due attention should be paid to conditions inside the factory regarding Hygiene, Lighting, Ventilation and Safety factors, the problem of fatigue in industrial workers etc., questions regarding the maintenance of canteens and of providing the workers with suitable housing must not be overlooked as one was as important as the other. The Committee when discussing this felt that one member of the Committee should be a person who has practical experience in the field of sanitary engineering and recommended in this connection the name of Professor K. Subrahmanyan, Professor of Sanitary Engineering, All-India Institute of Hygiene and Public Health, Calcutta.

10. The question regarding the propagation of knowledge connected with modern and most recent development of hazards in industry was discussed.

The Committee considered that in order to acquaint the Government, Factory owners and workers with work that has been done on the subject, the Indian Research Fund Association should be asked to finance the compilation of booklets describing the hazards in industry. One of the members pointed out that the Royal Institute of Chemistry publishes booklets on the subject.

INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS

1. (a) *The Indian Journal of Medical Research, Volume XXXII, 1944.*—Only two numbers of the Journal were published, and these appeared in May and October respectively. The new style of printing with narrower margins was continued in the interests of economy. The average size of each number was 126 pages of printed and tabular matter, with three plates. Vol. XXXII corresponded almost exactly with Vol. XXXI in size.

(b) *The Memoirs.*—No memoir was published with Vol. XXXII as no material was received which satisfied the requirement that the work done would be likely to aid the war effort.

(c) *Cost.*—The net cost of publication of Vol. XXXII (two numbers only) was Rs. 3,575/-.

2. (a) *The Indian Journal of Medical Research, Volume XXXIII, 1945.*—This volume will consist of two numbers only. The publication of the first number (May 1945) was delayed until August, although the typescripts were submitted to the printers in good time. The second number (October 1945) has not yet appeared but it is expected that it will not be more than one month late. Every effort is being made to ensure the appearance of the Journal on the dates due, and such delays as have occurred have been entirely the fault of the publishers. The May issue contained 176 pages and 9 plates, while the October number will consist of approximately 150 pages without any plates. The average for the two numbers of Vol. XXXIII will, therefore, be approximately 163 pages with 4 plates, which represents an increase in size of about 40 pages in each number as compared with Vol. XXXII.

(b) *The Memoirs :*—As no suitable material was received, no memoir was published. The advice of the Editor was sought as to the feasibility of bringing out a special tuberculosis number of the Journal or a special tuberculosis Memoir. There appears to be no reason why suitable papers on tuberculosis should not be accepted for publication in the Journal in the ordinary way. The opinion was expressed that if a sufficient number of papers on tuberculosis were submitted at any one time, it would be justifiable to publish a special number or a special memoir.

Distribution.—

Indian subscribers	 117	Same as for 1943 and 1944.	
Foreign subscribers	.. .. 94 =211	Figs. for 1945 not yet available.	
Exchange List	 187	"	
Free List	.. .. 126 =313	Many of the 72 addresses suspended during the war have now been resumed, but the exact number will be known next year.	
Voucher copies to advertisers, regn., make-up, police, specimen, propaganda, etc.	.. ..	40	approx.
Balance left with Publishers for sale	..	136	
Total Copies printed	..	700	

The exchange and free lists have been increased by the addition of three new addresses. Owing to the war, 72 foreign addresses on the distribution list were suspended, but some of these have now been resumed and it is anticipated that it will be possible to distribute the Journal to the remainder shortly. Similarly the number of publications received in exchange for the Indian Journal of Medical Research is already showing an increase, owing to receipts of journals from liberated countries.

3. *Distribution Lists.*—The distribution lists have not been revised since July 1938. It will be necessary to revise these lists and bring them up-to-date as soon as free communication with foregin countries is again possible.

4. *Number of copies printed.*—Only 700 copies of the Journal have been printed since July 1940 instead of the previous 900. This number is considered sufficient to meet all current demands. Vols. I-VIII of the Journal are out of print.

5. *Cost of printing the journal.*—The following statement shows the cost of production during the last five years :—

Cost of Printing.

Number	XXVII-XXVIII-XXIX 1940	XXIX-XXX 1941	XXX 1942	XXXI 1943	XXXII 1944
April	Rs. 5,137	2,052*	2,949*	May 2,633*	May 2,116*
July	Rs. 3,932*	3,531*	1,650*		
October	Rs. 4,717*	3,413*	2,413*	2,486*	2,752*
January	Rs. 3,571*	3,531*			
Totals	Rs. 17,357	11,527	7,012	5,199	4,868
Average pages	317	214	158	125	126

*700 copies only.

Recoveries.

April	Rs. 1,452	1,325	1,034	May 837	May 1,293
July	Rs. 1,451	1,325	946		
October	Rs. 963	837	1,518	880	
January	Rs. 963	837			
Totals	Rs. 4,829	4,324	3,498	1,717	1,293

6. *Net cost of Journal and Memoirs.*—The following statement shows the net cost of the production of the Journal and Memoirs during the last five years :—

Journal.

Number	1940	1941	1942	1943	1944
April	Rs. 3,685	727	1,915	May 1,796	May 823
July	Rs. 2,481	2,206	704		
October	Rs. 3,754	2,576	895	1,606	2,752
January	Rs. 2,608	1,694			
Totals	Rs. 12,528	7,203	3,514	3,402	3,575

Memoirs.

No. 33	Rs.	1,565			
No. 34	Rs.	1,627			
Totals	Rs.	3,192			
Grand Totals	Rs. 12,528	10,395	3,514	3,402	3,575

For convenience of accounting, all receipts are credited to the Journal account, and the figures given for the Memoirs are the actual cost of production.

The net cost during 1944, after deduction of receipts, was Rs. 3,575. All publishers' bills for 1944 were submitted in time to make payments within the financial year.

7. *Financial position for the year 1945-46.*—The budget for the year provided for Rs. 7,000/- to meet the cost of two half-yearly numbers of the Journal and Rs. 2,000/- for any Memoir which might be published. The following is the estimated expenditure :—

Volume XXXIII, No. 1 (May 1945) } No. 2 (Oct. 1945) } at Rs. 3,500 each	Rs. 7,000 estimated.
No Memoir was published	Nil
Total	Rs. 7,000

8. *General.*—During the year the editorial policy of the Journal has been revised on the recommendation of the Scientific Advisory Board. The Editorial Board has been enlarged to include 25 referees. A note for the guidance of members of the Board was prepared by the Editor and circulated to all concerned.

It is for consideration whether the Journal should now resume the publication of four numbers each year as was the regular practice before the war. Although it may be anticipated that with the cessation of hostilities research in India will soon be revived, it cannot be foreseen that the results of these researches are likely to be prepared and submitted for publication before the end of 1946, when acceptances will close for Vol. XXXIV of the Journal. In the Editor's opinion, therefore, there is not a strong case for the immediate resumption of quarterly publication.

All of the papers published in Vol. XXXIII were submitted by workers in India or were the results of investigations carried out in India. Of the 37 articles published in this volume, 16 were on nutrition, 6 on bacteriology, 5 on biochemistry, 3 on pharmacology ; 3 on haematology and 2 on rabies.

The size of the Journal has been considerably reduced since 1944 owing partly to the new style of printing adopted, partly to a reduction in the number of papers received for publication and partly to the efforts made by contributors to reduce their papers to minimum size.

9. *Budget estimate for 1946-47.*—Pending a decision as to the resumption of quarterly publication, the Editor proposes to publish only two numbers of the Journal during 1946. These will appear in May and October as in recent years. It is proposed to print only 700 copies of each number. Provision is also being made for the publication of one Memoir should suitable material be received. The estimated cost of Vol. XXXIV is :

2 half-yearly issues of the Journal at Rs. 3,500 each	..	Rs. 7,000
1 Memoir	..	Rs. 2,000
Total	..	Rs. 9,000

An additional allotment will be required should it be decided to publish a special tuberculosis number of the Journal or a special tuberculosis Memoir. This would amount to approximately Rs. 3,500/-.

The cost of individual numbers of the Journal has not been altered, but the annual subscription has been reduced from Rs. 16/- to Rs. 8/- so long as only two half-yearly numbers are published.

Publications of medical research under the auspices of the Indian Research Fund Association during the year 1945.

A. *Indian Journal of Medical Research and Indian Medical Research Memoirs.*

1. BASHIR AHMAD, DUTT, T. R., and NARANG, B. D.—Studies on Calcium and Phosphorus Matabolism. Part I. Calcium, Phosphorus and Phytin Contents of the Diets of Kangra Valley and their relation to Nutrition (Vol. 33, No. 1).
2. BASHIR AHMAD, SEHRA, K. B. and SWAROOP, S.—Studies on Calcium and Phosphorus Metabolism. Part II. Blood-Serum Calcium, Phosphorus and Phosphatase of the Population of Kangra Valley (Vol. 33, No. 1).
3. BOSE, S. M. and BANERJEE, B. N.—Studies on the Destruction of Vitamin A in Shark-Liver Oil. Part V. Effect of some Anti-oxidants on the Stabilization of Vitamin A (Vol. 33, No. 2).
4. CHOPRA, I. C., KOHLI, J. D. and HANDA, K. L.—*Inula royleana* D.C. : Its Chemistry and Pharmacological Action (Vol. 33, No. 1).
5. CHOPRA, I. C., KOHLI, J. D. and HANDA, K. L.—Chemistry and Pharmacology of some common indigenous remedies (Vol. 33, No. 1).

6. GHOSH, S., GUPTA, J. C. and KAHALI, B. S.—Assay of Anthracene Purgatives by the Estimation of the Content of Hydroxymethyl-anthraquinones. Part I. Rhubarb (Vol. 33, No. 1).
7. KHANOLKAR, V. R.—The Susceptibility of Indians to Cancer (Vol. 33, No. 2).
8. MAJUMDER, D. N. and DAS GUPTA, C. R.—Haematological Studies in *Silenus* (*macacus*) *rhesus*. Part II. Nature of Malarial Anaemia in Monkeys (Vol. 33, No. 1).
9. MASON, ELEANOR D., THEOPHILUS, FLORENCE and FRIMODT, MOLLER, J.—The Influence of Butter Fat in the Absence or Presence of Casein on Growth in Young Rats on a Rice Diet (Vol. 33, No. 2).
10. MITRA, K., and MITTRA, H. C.—Biological value of Proteins from Muscle Meat of Cow, Buffalo and Goat (Vol. 33, No. 1).
11. MITRA, K., and MITTRA, H. C.—Food Value of Further Batch of Edibles. Estimated by Chemical Methods (Vol. 33, No. 1).
12. PANJA, G.—Aetiology and Treatment of *Ulcus tropicum* (Vol. 33, No. 1).
13. PATWARDHAN, V. N., CHITRE, R. G., and SUKHTANKAR, D. R.—Studies in Calcium and Phosphorus Metabolism. Part VII. The Ionic Products of Calcium Phosphates in Experimentally Induced Vitamin D Deficiency (Vol. 33, No. 2).
14. RAO, S. DATTATREYA.—Studies on the Destruction of Vitamin A in Shark-Liver Oil. Part III. (Relation between Vitamin A Destruction and Peroxide Number (Vol. 33, No. 1).
15. RAO, S. DATTATRYEA—Studies on the Destruction of Vitamin A in Shark-Liver Oil. Part IV. Catalytic Activity of Metals (Vol. 33, No. 1).
16. RAO, S. SUNDAR—Filarial Infection in Dhamda (Drug District, C.P.) due to *Wuchereria malayi* (Vol. 33, No. 1).
17. SEHRA, K. B. and BASHIR AHMAD.—Studies on Calcium and Phosphorus Metabolism. Part III. Hepatic Efficiency and Rickets. (Vol. 33, No. 1).
18. SHOURIE, K. L.—An outbreak of Lathyrism in Central India (Vol. 33, No. 2).
19. SREERAMAMURTHY, V. V.—Investigations on the Nutritive Value of Tapioca (Manihot utilissima) (Vol. 33, No. 2).

B. *Journal of the Malaria Institute of India.*

1. COVELL, G.—False economy in the planning and conduct of antimalaria campaigns (Vol. VI, No. 2).
2. MOHAN, B. N.—Details of the procedure adopted in maintaining a laboratory colony of *A. fluviatilis* (Vol. VI, No. 1).
3. PAL, RAJINDAR—On the bionomics of *A. culicifacies* Giles. Part II. The ecology of the immature stages (Vol. VI, No. 1).
4. PAL, RAJINDAR—The bionomics of *A. culicifacies* Giles. Part III. The behaviour of adults (Vol. VI, No. 2).
5. PAL, RAJINDAR—On the bionomics of *A. culicifacies* Giles. Part IV. Hypothesis of the races of *A. culicifacies* (Vol. VI, No. 2).

6. YACOB, M. and SATYA SWAROOP—Investigation of long term periodicity in the incidence of epidemic malaria in the Punjab (Vol. VI, No. 1).

C. *List of papers published by workers under the I.R.F.A. in journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

1. DHARMENDRA and SANTRA, I.—Epidemiological leprosy surveys in various parts of India (Leprosy in India Vol. XVII, No. 1).
2. DHARMENDRA and CHATTERJI, S. N.—Trigeminal neuritis simulating leprosy (Leprosy in India Vol. XVII, No. 2).
3. DHARMENDRA and RAJAGOPALAN, S.—Some aspects of chemotherapy in leprosy (Leprosy in India Vol. XVII, No. 3).
4. DHARMENDRA—Leprosy in Bengal (Leprosy in India Vol. XVII No. 4).
5. DHARMENDRA—Popular lecture on leprosy—A booklet illustrated with lantern slides. (Published by B. E. L. R. Association, Indian Council, New Delhi).
6. GHOSH, L. M. and DEY, N. C.—Studies in Ringworm Part II. (I.M.G. Vol. LXXX. p. 381).
7. GHOSH, L. M. and PANJA, D.—A case of Rhinoscleroma (I.M.G. Vol. LXXX. October).
8. SEN GUPTA, P. C.—Complement-fixation test with Witebsky, Klengenstein, Kuhn (WKK) or similar antigens. A modified technique (I.M.G. Vol. LXXX, p. 396).
9. SEN GUPTA, P. C.—4 : 4′ Diamidino-diphenyl-ether in the treatment of Indian Kala-azar (I.M.G. Vol. LXXX).
10. SEN GUPTA, P. C. and CHAKRAVARTY, N. K.—The treatment of kala-azar with sodium antimony gluconate (I.M.G. Vol. LXXX).
11. SEN GUPTA, P. C. and CHAKRAVARTY, N. K.—The treatment of cancrum oris complicating kala-azar. (I.M.G. Vol. LXXX).
12. SUNDAR RAO, S.—Filariasis in Bengal (I.M.J. Vol. 39, No. 6).
13. SUNDAR RAO, S—Treatment of filarial infection and diseases (I.M.J. Vol. 39 No. 7).
14. COVELL, G.—Malaria control in South West Pacific. (J. United Ser. Institution Ind. January 1945).
15. JASWANT SINGH and BHATTACHARJI, L. M.—Note on the J.S.B. method of staining malaria parasites (I.M.G. Vol. LXXX. No. 3 p. 159).
16. RAJINDRA PAL—Nephrocytes in some culicine-Diptera (Ind. J. Ent. Vol. VI pp. 143-148).
17. COVELL, G.—Malaria Problem in Rural India (Perspective).
18. COVELL, G.—Note on the part played by the Malaria Institute of India in the War Effort. (Indian Information).
19. COVELL, G.—Note on the history and activities of the Malaria Institute of India (Ind. Med. Rev.).
20. COVELL, G.—Role of D.D.T. in combating disease among civilian population (Ind. Hlth. Gaz.).
21. PURI, I. M.—Review of work on medical entomology in India from 1938 onwards (Ind. Med. Rev.).

The following enquiries and budget allotments were recommended to the Governing body by the Scientific Advisory Board at its meeting held on the 30th November and 1st December, 1945.

	Enquiries.	Budget allotments. Rs.
	Cholera.	
1.	Cholera treatment unit under the Director, School of Tropical Medicine, Calcutta	30,417
2.	Cholera treatment unit under the Director, King Institute, Guindy, Madras	23,684
3.	Enquiry to prepare monospecific high titre cholera sera under the Director, King Institute, Guindy, Mardas	3,000
4.	Enquiry on cholera under the Director, Central Research Institute, Kasauli	29,894
	Malaria.	
5.	Malaria Institute of India, Research Section, Delhi ..	42,142
6.	Southern India Branch of the Research Section of the Malaria Institute of India at the Pasteur Institute, Coonoor ..	14,933
7.	Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi	31,099
8.	Mammalian malaria enquiry under the Director, Central Research Institute, Kasauli	55,914
9.	Filariasis enquiry under Dr. Sundar Rao at the School of Tropical Medicine, Calcutta	15,848
10.	Enquiry on adult *Anopheles stephensi* under the Director, School of Tropical Medicine, Calcutta	8,016
11.	Enquiry on the control of sandflies *Phlebotomus argentipes* by spraying with D.D.T. under Dr. D. N. Roy, Professor of Medical Entomology, School of Tropical Medicine, Calcutta ..	8,500
	Nutrition.	
12.	Nutrition Research Laboratories, Coonoor	93,857
13.	Additional nutrition enquiry under the Director, Nutrition Research Laboratories, Coonoor	26,658
14.	Ascorbic acid enquiry at the University College of Science and Technology, Calcutta	6,514
15.	Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay	2,316
16.	Nutrition research unit under Dr. K. P. Basu at Dacca University, Dacca	17,787
17.	Nutrition research unit under Dr. V. N. Patwardhan at the Seth G. S. Medical College, Bombay	16,953

	Enquiries.	Budget allotments Rs.
18.	Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the Laboratories of the Board of Science and Industrial Research, University Buildings, Delhi ..	5,478
19.	Enquiry on the chemical nature and nutritional availability of food iron at the University College of Science and Technology, Calcutta	3,676
20.	Enquiry on poor rice diets and food preparations in common use in South India under Miss D. Pearson at the Women's Christian College, Madras	3,718
21.	Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary proteins to blood and muscle haemoglobin under Professor M. Damodaran at the University Biochemical Laboratory, Triplicane, Madras ..	3,803
22.	Enquiry entitled "Preparation of antirachitic substances" under Professor B. V. Bhide at the Maharajah Pratap Singh Chemical Laboratory, S. P. College, Poona	3,932
	Leprosy.	
23.	Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.	13,379
	Plague.	
24.	Plague researches under the Director, Haffkine Institute, Bombay	18,000
	Clinical Research.	
25.	Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay	8,633
26.	Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	10,712
27.	Enquiry on Naga sore under Dr. S. R. Pandit, Director, Pasteur Institute, Shillong	9,136
28.	Enquiry into hypropoteinaemia and other conditions associated with malnutrition under Dr. John Lowe at the School of Tropical Medicine, Calcutta	5,001
29.	Enquiry into erythroblastosis foetalis and its association with the Rh factor under Lieut.-Colonel S.D.S. Grevel, I.M.S., Imperial Serologist to the Government of India at the School of Tropical Medicine, Calcutta	3,472
30.	Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta	12,712
31.	Enquiry into infections of the feet under Rao Bahadur Dr. M. G. Kini, Superintendent, Stanley Medical Hospital, Madras ..	5,150

	Enquiries.	Budget allotments. Rs.
32.	Enquiry on cirrhosis in animals and its bearing on the aetiology of infantile biliary cirrhosis under Dr. T. Bhaskara Menon, Professor of Pathology, Andhra Medical College, Vizagapatam	4,272
33.	Enquiry on infantile cirrhosis under Dr. P. Kutumbiah, Principal, Stanley Medical College, Madras	3,612
34.	Dental research unit under Dr. C. D. Marshall Day, Principal, Demontmorency College of Dentistry, Lahore	10,252
35.	Enquiry on the relationship between the measurements of vertebrae and body size of Indians under Dr. R. G. Ginde at the Seth G. S. Medical College, Bombay	2,864
36.	Enquiry into causes of blindness under Captain E. J. Somerset, I.M.S., Professor of Ophthalmology at the Medical College, Calcutta	3,212
	Pharmacology.	
37.	Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S. (R) at the Drug Research Laboratory, Jammu, Kashmir	6,107
	Maternity and Child Welfare.	
38.	Enquiry into anaemia in pregnancy in the Singur health unit under Dr. J. Orkney, W.M.S. at the All-India Institute of Hygiene and Public Health, Calcutta	7,380
39.	Enquiry into the pelvic type and its relationship to body build in Indian women under Drs. S. K. Basu, S. Mitra and S. Mukerji at the Carmichael Medical College, Calcutta	2,500
40.	Enquiry into the bearing of pre-mature and immature births on infant mortality under Dr. B. C. Das Gupta, Executive Health Officer, Bombay Municipality, Bombay	10,872
	Other Researches.	
41.	Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	10,851
42.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	10,220
43.	Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay ..	5,880
44.	Enquiry on the action of snake venoms and other constituents on the biochemical activity of tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta	3,395
45.	Enquiry on typhus under Dr. S. R. Savoor at the Haffkine Institute, Bombay	13,387
46.	Enquiry on Kala-azar under Dr. P. C. Sen Gupta at the School of Tropical Medicine, Calcutta	6,140

	ENQUIRIES.	Budget allotments Rs.
47.	Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta	6,762
48.	Enquiry into methods for the manufacture of penicillin and other problems connected with it under the Director, Haffkine Institute, Bombay	17,277

GRANTS.

(i)	Office of the Secretary, Indian Research Fund Association ..		36,096
(ii)	Staff employed in the office of the Accountant General, Central Revenues for I.R.F.A. work		6,629
(iii)	I.R.F.A. publications to be published during 1946-47 ..		4,000
(iv)	Travelling allowance of the members of the Governing Body and Scientific Advisory Board and Advisory Committees of the I.R.F.A.		8,000
(v)	Contribution towards the cost of two professorships at the School of Tropical Medicine, Calcutta		61,356
(vi)	Contribution towards the cost of a plague enquiry at Coonoor in the Nilgiris District under the Director of Public Health, Madras		6,000
(vii)	Contribution towards the cost of the leprosy campaign in Madras to Dr. R. G. Cochrane, Honorary Director, Leprosy Campaign, Vellore		2,500
(viii)	Contribution to the Imperial Institute of Entomology, London		1,350
(ix)	Contribution towards the Bureau of Hygiene and Tropical Diseases, London		2,025
(x)	One research fellowship of the value of Rs. 150/- p.m. ..		1,800
(xi)	Microfilm unit at the Central Research Institute, Kasauli ..		13,118
(xii)	Indian Journal of Medical Research and Memoirs		
	(a) Printing and publication charges of the Journal	10,500	
	(b) Printing & publication charges of the Memoirs	2,000	
	(c) Editor's office	5,475	17,975
(xiii)	I.R.F.A. Library at the Central Research Institute, Kasauli		2,278
(xiv)	Pay (including special pay, overseas pay & allowances) and contributions towards passage leave salary and pension of contributory provident fund of the officers of the Medical Research Department under the Association		1,50,000
(xv)	Travelling allowance and certain contingent expenses for Medical Research Department Officers employed under the I.R.F.A. and attached to Central or Provincial Laboratories		10,000

	Grants.	Budget allotments Rs.
(xvi)	Pay and travelling allowance of Junior Research Worker ..	10,180
(xvii)	Clerical assistance at the School of Tropical Medicine, Calcutta	5,355
(xviii)	Clerical assistance at the All-India Institute of Hygiene and Public Health, Calcutta	1,284
(xix)	Travelling allowance for subordinate I.R.F.A. workers on transfer	3,000
(xx)	I.R.F.A. contribution and interest towards the Contributory Provident Fund	15,000
(xxi)	Miscellaneous unforseen expenditure	35,000
(xxii)	Imprest advance for enquiries	12,030

30 JUN 1947

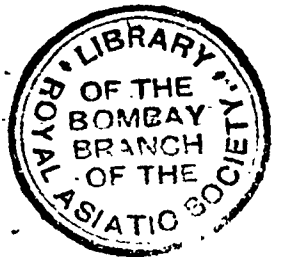
REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

1st January to 31st December, 1946.



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The Scientific Advisory Board consisted of the following :—

Chairman :

Lieut.-General J. B. Hance, C.I.E., O.B.E., K.H.S., I.M.S., Director-General Indian Medical Service (1st January to 11th March, 1946).

Lieut.-General R. Hay, C.I.E., K.H.P., I.M.S., Director-General, Indian Medical Service (12th March to 31st December, 1946).

Members :

Lieut.-Colonel C. A. Bozman, I.M.S., Offg. Public Health Commissioner with the Government of India (1st January to 3rd April, 1946). *Secretary.*

Colonel E. Cotter, C.I.E., V.H.S., I.M.S., Public Health Commissioner with the Government of India, (4th April to 31st December, 1946). *Secretary.*

Lieut.-Colonel H. W. Mulligan, I.M.S., Director, Central Research Institute, Kasauli (1st April to 6th July, 1946 and 6th November to 31st December, 1946).

Lieut.-Colonel W. J. Webster, I.M.S., Offg. Director, Central Research Institute, Kasauli (1st January to 31st March, 1946 and 7th July to 5th November, 1946).

Lieut.-Colonel C. L. Pasricha, I.M.S., Director, School of Tropical Medicine, Calcutta (1st January to 22nd February, 1946 and 13th August to 31st December, 1946).

Dr. J. Lowe, M.D., Offg. Director, School of Tropical Medicine, Calcutta (23rd February to 7th July, 1946).

Dr. D. N. Roy, M.D., D.T.M., Offg. Director, School of Tropical Medicine, Calcutta (8th July to 12th August, 1946).

Lieut.-Colonel Sir Sahib Singh Sokhey, I.M.S., Director, Haffkine Institute, Bombay (1st January to 5th June, 1946 and 23rd November to 31st December, 1946).

Major J. R. Dogra, I.M.S., Offg. Director, Haffkine Institute, Bombay (6th June to 22nd November, 1946).

Dr. C. G. Pandit, O.B.E., M.B.B.S., Ph.D., D.P.H., D.T.M., Director, King Institute, Guindy, Madras.

Major-General Sir Gordon Covell, C.I.E., K.H.P., I.M.S., Director, Malaria Institute of India, Delhi.

Lieut.-Colonel C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. Bashir Ahmad, M.Sc., Ph.D. (Lond.), F.N.I., Assistant Director, Council of Scientific and Industrial Research, New Delhi.

Dr. A. N. Goyle, M.D., Ph.D., Professor of Pathology, King Edward Medical College, Punjab, Lahore.

Dr. B. C. Guha, D.Sc., F.N.I., Chief Technical Adviser, Government of India, Department of Food, New Delhi.

Professor S. W. Hardikar, M.D., M.R.C.P., " Abhas " Hardikar Bagh, Himayatnagar, Hyderabad (Deccan).

Dr. V. R. Khanolkar, B.Sc. (Lond.), M.D. (Lond.), Director of Laboratories, Tata Memorial Hospital, Bombay.

Dr. Jivraj N. Mehta, M.D.(Lond.), M.R.C.P., Khambatta Hill, 16 Altamont Road, Bombay, 26.

Dr. V. N. Patwardhan, M.Sc., Ph.D., Director, Nutrition Research Laboratories, Coonoor.

Co-opted member :

Dr. Muktha Sen, Professor of Maternity & Child Welfare, All-India Institute of Hygiene & Public Health, Calcutta.

Composition of Advisory Committees for the year 1947.

1. *Cholera.*

Lieut.-Colonel H. W. MULLIGAN. (*Chairman*).
Colonel E. COTTER.
Dr. B. B. DIKSHIT
Major J. H. GORMAN.
Dr. C. G. PANDIT.
Dr. S. R. PANDIT.
Lieut.-Colonel C. L. PASRICHA.
Rao Bahadur Dr. K. C. K. E. RAJA.
Dr. D. L. SHRIVASTAVA.
Lieut.-Colonel Sir SAHIB SINGH SOKHEY.
Lieut.-Colonel M. L. AHUJA. } (*Joint Secretaries*).
Dr. K. V. VENKATRAMAN. }

2. *Malaria.*

Major-General Sir GORDON COVELL. (*Chairman*).
Lieut.-Colonel M. K. AFRIDI.
Dr. B. ANANTHASWAMY RAO.
Rai Bahadur Dr. A. C. BANERJEA.
Bt.-Colonel Sir RAM NATH CHOPRA.
Dr. M. O. T. IYENGAR.
Lieut.-Colonel H. W. MULLIGAN.
Dr. P. KUMARAN NAIR.
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Lieut.-Colonel JASWANT SINGH. (*Secretary*).

3. *Nutrition.*

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Professor S. P. AGHARKAR.
Dr. BASHIR AHMAD.
Dr. K. P. BASU.
Dr. B. C. GUHA.
Lieut.-Colonel C. M. NICOL.
Dr. M. V. RADHAKRISHNA RAO.
Dr. G. SANKARAN.
Sir JOHN SARGENT.
Sir HERBERT STEWART.
Mr. GRAHAME WILLIAMSON.
Dr. V. N. PATWARDHAN. (*Secretary*).

4. *Plague.*

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Dr. P. V. GEORGE.
Dr. A. N. GOYLE.
Dr. C. G. PANDIT.
Dr. S. R. PANDIT.

Dr. M. SHARIF.
Dr. K. V. VENKATRAMAN.
Dr. P. M. WAGLE.
Lieut.-Colonel Sir SAHIB SINGH SOKHEY. (*Secretary*).

5. *Clinical Research.*

Major A. T. ANDREASEN. (*Chairman*).
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Dr. M. N. DE.
Dr. A. N. GOYLE.
Dr. S. W. HARDIKAR.
Lieut.-Colonel JASWANT SINGH.
Dr. M. G. KINI.
Dr. P. KUTUMBIAH.
Dr. JIVRAJ N. MEHTA.
Dr. J. C. PATEL.
Lieut.-Colonel A. SACHS.
Dr. M. A. SHAH.
Major F. A. B. SHEPPARD.
Captain E. J. SOMERSET.
Dr. B. B. YODH.
Dr. V. R. KHANOLKAR. (*Secretary*).

6. *Rabies.*

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Dr. S. R. PANDIT.
Dr. R. SANJIVA RAO.
Mr. J. F. SHIRLAW.
Dr. N. VEERARAGHAVAN.
Dr. A. G. BROOKS. (*Secretary*).

7. *Leprosy.*

Dr. C. G. PANDIT. (*Chairman*).
Lieut.-Colonel A. N. CHOPRA.
Dr. R. G. COCHRANE.
Dr. P. V. GHARPURE.
Dr. V. R. KHANOLKAR.
Dr. JOHN LOWE.
Lieut.-Colonel H. W. MULLIGAN.
Dr. DHARMENDRA. (*Secretary*).

8. *Filariasis.*

Major-General Sir GORDON COVELL. (*Chairman*).
Dr. R. G. DHAYAGUDE.
Dr. B. B. DIKSHIT.
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Dr. A. K. MUKERJI.
Lieut.-Colonel H. W. MULLIGAN.

Lieut.-Colonel C. L. PASRICHA.
Major F. A. B. SHEPPARD.
Major R. SENIOR WHITE.
Dr. C. G. PANDIT. (*Secretary*).

9. *Industrial Health.*

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Mr. S. P. JAIN.
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Dr. M. G. KINI.
Mr. N. S. MANKIKER.
Dr. J. M. ORKNEY.
Dr. J. N. RAY.
Major J. SEN.
Professor K. SUBRAHMANYAN.
Dr. M. N. RAO (*Secretary*).

10. *Maternity and Child Welfare.*

Dr. J. JHIRAD. (*Chairman*).
Dr. A. BENJAMIN.
Miss M. CRAIG.
Dr. J. M. ORKNEY.
Dr. N. A. PURANDARE.
Dr. MUKTHA SEN.
Dr. S. PANDIT. (*Secretary*).

The Board held its meetings on the 24th July, 1946 and on the 9th, 13th and 14th December, 1946. At its annual meeting held in December, 1946 the Board confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers and decided on the co-option of experts for its annual meeting. It also considered in detail the programme of research for the year 1947-48 and recommended the programme given on pages 226 to 232 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1946.

The researches, which were carried out during the year under report were recommended by the Board at its meeting held in New Delhi on the 30th November and 1st December, 1945, and were approved by the Governing Body of the Indian Research Fund Association at its meeting held in New Delhi on the 16th March, 1946.

CHOLERA.

1. Cholera treatment unit under the Director, School of Tropical Medicine, Calcutta.

As there was very little cholera during the year no extensive series of trials with sulpha group of drugs were carried out.

(A) *Field trials* :—(I) At Baruipara, Domjur, in Howrah district, 12 miles from Calcutta, a small outbreak of cholera occurred in March, 1946. Twenty-one bacteriological proven cases of cholera were treated under village home conditions. The selection was on alternate case basis. Ten of the patients received sulphaguanidine in 3 gm. doses every three hours for 3 days and twice a day for a further 3 days. Eleven patients received a placebo. There were no deaths in the sulphaguanidine series whereas in the control series there were 2 deaths. Sulphaguanidine was used as a prophylactic (in doses of 2 gm. a day) in 23 close contacts of cholera. There was no cholera amongst individuals receiving sulphaguanidine whereas in another group of contacts not receiving sulphaguanidine there were two cases of cholera.

(II) At Nabadwip, in Nadia District, 64 miles from Calcutta, an outbreak of 160 cases occurred in December, 1946. A treatment unit reached there during the height of the epidemic and a series of patients were treated. Sulphaguanidine was given according to the dosage outlined under (I). The results were :—

Treated with sulphaguanidine 25, deaths 6 or 24 per cent.
Given a placebo 25, deaths 12 or 48 per cent.

Seven contacts of cholera patients suffering from diarrhoea and vomiting (4 patients) were given sulphaguanidine on the day of onset. They all improved within 2 or 3 days.

(III) Workers went to Chittagong in anticipation of an outbreak of cholera following the heavy flood there in July, 1946. Unfortunately there was no cholera there.

(B) *Therapeutic trials in Calcutta* :—(I) Sulphaguanidine and sulphasuxidine were used in parallel series of cases. The total daily dose was 15 gms., the control series receiving a placebo. The results are shown below :—

	Total No.	No. of deaths:	Mortality.
Sulphaguanidine series	192	8	4·1
Sulphasuxidine series	190	7	3·7
Control series	189	7	3·7

(II) *Sulphadiazine* :—In another group of cases sulphadiazine and sulphaguanidine were used. During this period cholera cases were atypical and many were intestinal fluxes due to other causes than cholera. The results are shown below :—

	Total No.	No. of deaths	Mortality
Sulphaguanidine series (15 gms. a day)	111	2	1·8
Sulphadiazine series (5 gms. a day)	99	2	2·02
Control series	103	1	0·97

(C) *Laboratory* :—A total of 2,134 stools from 1,094 cases of clinical cholera were examined during the year. Of these 320 or 29·2 per cent were bacteriologically proven cholera cases. During the epidemic period (March, April and May) 844 samples of stools from 472 clinical cases were examined and 255 cases were bacteriologically proven cholera. Out of 320 strains isolated 282 or 88·1 per cent were of the Ogawa subtype and 38 or 11·9 per cent were of the Inaba subtype.

Chloride in cholera stools :—100 samples of typical rice watery cholera stools were examined—the average chloride content in the stools was 0·431 grammes and the average pH 7·8.

2. Cholera treatment unit under the Director, King Institute, Guindy, Madras.

The period under report was marked by a low incidence of cholera in the Presidency, and no field work could be undertaken. The field unit was stationed at the King Institute, Guindy.

The experimental production of cholera in monkeys has been the subject of study during this period. The immunity of the animal to cholera was assumed to reside in the intestinal mucosa and attempts to break down the immunity were made by one of two procedures.

(a) Injection of 20 ml. of distilled water at 56° C into the lumen of the small intestine after a laparotomy.

(b) Irradiation of the abdominal wall of the monkey by deep X-rays, the dose being 600 r, corresponding to the erythema dose in human beings.

The first procedure was adopted in the case of three animals while the latter was adopted in eight animals. Two animals, however, became sickly after X-ray dose and died during the course of the week.

The administration of the vibrios was carried out by opening the abdominal cavity and injecting a saline suspension of *V. cholerae* inside the small intestines, the opening being subsequently closed by a purse string suture. After suturing up the wound, the abdominal wall was well bandaged and protected by a metal plate.

The time of administration of *V. Cholerae* depends upon the nature of the preparatory method adopted. When distilled water at 56° C was used in the preliminary treatment, *V. cholerae* was administered after an interval of 24 hours.

In one case, however, the vibrio administration was made ten minutes after the injection of hot water. In the case of the irradiated animals the vibrios were administered seven days after the X-ray application—a period when the effect of the X-rays is supposed to be maximal.

The results of the experiments have so far been inconclusive. The results obtained with the irradiated animal were definitely better than that obtained with those where hot water was used to destroy the protective mucosal epithelium.

A pronounced diarrhoea was noticeable in five animals (all irradiated previously) which commenced 48 hours after the administration of the vibrios. In three of these animals the vibrio could not be isolated from the faeces. The faecal matter in these animals were semi-solid in consistency and sometimes quite watery. The quantity was never quite large, the maximum single motion never exceeding an ounce in quantity. The animal did not exhibit any other symptom and recovery resulted in the course of the week. Isolation of the vibrios was impossible three days after the onset of the diarrhoea.

A post mortem examination of all but one animal was done. There was no characteristic change beyond a slight congestion of the omentum. Stained sections of the small intestines revealed areas where the mucosal lining was discontinuous.

Attempts were also made to infect guinea-pigs by oral administration of *V. cholerae*. The precedure adopted in the case of guinea-pig was to administer 2 spaced doses of sterile ox-bile followed after an interval by morphia and atropine and oral administration of a culture of *V. cholerae* preceded by an alkaline drink. The stools following the treatment were generally solid and occasionally semi-solid. No obvious diarrhoea could be induced.

14 guinea-pigs were used in these experiments and in only one animal could *V. cholerae* be isolated on the 3rd day after the infective dose, and isolation could be possible till the 5th day after which it disappeared.

3. Enquiry to prepare monospecific high titre cholera sera under the Director, King Institute, Guindy, Madras.

The preparation of monospecific agglutinating sera for Inaba and Ogawa types of *V. cholerae* was continued during the period under report, and the demands made by other workers (which have been quite small) have been met. 300 ml. of Mono-specific Ogawa 'O' serum and 35 ml. of Mono-specific Inaba 'O' serum are still available for distribution. A further 750 ml. of Inaba serum is under processing.

While the prepartion of highly specific monovalent Ogawa 'O' serum has been relatively easy, certain difficulties were met with in the case of Inaba. Initial high titres were easily obtained but absorption with the Ogawa strains used for the purpose has tended to reduce the homologous titre considerably. A systematic examination of a collection of Inaba and Ogawa strains has been undertaken with a view to the selection of suitable strains for immunisation and absorption. Absorption with specific fractions of the vibrio antigens has also been planned.

4. Enquiry on cholera under the Director, Central Research Institute, Kasauli.

The cholera enquiry now being carried on at the Central Research Institute, Kasauli, was begun in April of this year. Broadly it is concerned with questions regarding the substance and variability of the vibrios which may have a bearing on the efficiency of the vaccine used in cholera prophylaxis.

During the years preceding the recent war a considerable amount of serological and biochemical work on *Vibrio cholerae* and related vibrios was done by workers in different countries, but though important results were achieved, many of the observations made in these several researches remain incompletely correlated. One of the primary objects of the present study is to reduce so far as possible the various observations to a common scheme and to fill in obvious gaps in knowledge by further work. To take a case in point: during the latter part of their collaboration Linton *et al.* in this country separated from a vibrio strain isolated in the early stages of an epidemic a glucolipid fraction which they thought might conceivably be related to epidemicity; it now becomes of interest to identify this material with, or to differentiate it from, the "antigene glycolipide" of French and Roumanian authors and the "Specific antigen" of Bruce White, regarded by those workers as the essential agglutinating antigen of the cholera vibrio. Steps have been taken to accumulate material for the requisite comparative serological and biochemical studies, though difficulties are being encountered in obtaining preparations in the quantities desired.

A study is also being made of the so-called β-fraction of Bruce White, believed by that author to be an essential constituent of the antigen of the rough vibrio but with regard to the chemical composition of which (beyond the statement that it is a carbohydrate-containing non-protein, possibly a polysaccharide) he gave no adequate definition. Stocks of this material have been and are being prepared for examination.

With the agreement of the Secretary, Indian Research Fund Association, and of the Director of the Central Research Institute, Kasauli, Mr. P. Bruce White, now paying a short visit to India on behalf of the Medical Research Council and at the invitation of the Indian Research Fund Association, joined Dr. Shrivastava in mid-November and these workers have together taken up, among other things, a study of the closer relationships of the subtypes of the cholera vibrio. It is yet too soon to make a precise statement of the findings but it may be said that the results to date, though very incomplete and in need of close scrutiny, appear at the moment to be of a considerable interest.

MALARIA.

5. Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.

Work on the preparation of DDT emulsion concentrates has been continued. The object has been to prepare concentrated solution miscible with water in very high dilutions using medium kerosene extract (MKE) and turpentine as solvents. Various emulsifiers have been tried and an emulsion concentrate containing 35 per cent DDT has been prepared with MKE as a solvent. This emulsion concentrate, unlike the turpentine-toluene emulsion concentrate, is not highly inflammable. The different emulsions prepared have been subjected to various laboratory and field tests.

As preliminary experiments had shown that DDT suspension in water, when applied as a residual spray against adult mosquitoes, is as effective as DDT solutions or emulsions, various methods of preparing a suitable suspension were tried. A method of preparing a suspension has been evolved by which a 50 per cent suspension concentrate can easily be prepared using very cheap ingredients. Work on these lines is being continued with the object of developing a stock suspension of greater concentration.

For the application of indoor residual spray a stirrup pump fitted with a lance provided with a wheel-cock at one end and a fine nozzle with an orifice 1/32–1/64 inch in diameter at the other, has been found more satisfactory than any other type of spraying equipment. The output of such a nozzle is 8 to 12 oz. per minute and it takes one unit (two men operating one stirrup pump) about 12 minutes to spray a room of 1,000 square feet wall surface, spraying 2-3 c.c. of spray per square foot.

A simple pressure relief valve has been devised. When this is fitted near the bucket end of the hose, there is very little likelihood of the latter bursting while the pump is in use, or in the event of blockage of the nozzle. A model with a pressure relief valve forming an integral part of the main body has been found more satisfactory than any other type of stirrup pump. A relief valve fitted to the lower end of the pump is more satisfactory than one fitted to the hose a little away from the pump, because in the former any fluid escaping from the valve does not spill outside the bucket and moreover tends to keep the fluid in the bucket in constant motion. A special attachment has also been fitted to the stirrup pump which constantly agitates the fluid in the bucket when the pump is in operation.

A number of experiments have been carried out in the laboratory and in the field to test the comparative efficacy of different doses of DDT solution, emulsions and suspensions, and of gammexane solutions as residual sprays.

For experiments in the field at Delhi as well as at Quetta in Baluchistan, a number of villages were sprayed with different types and dilutions of solutions, emulsions and suspensions of DDT. At least two catching stations were earmarked in each section of the village under trial and attempts were made to collect the total mosquito population in each. This was done by closing all apertures and spraying the room with a 0·02 per cent solution of pyrethrins in kerosene and collecting mosquitoes from white sheets covering the floor.

(I) Delhi.

(a) *Turpentine emulsion and turpentine-toluene emulsion (army emulsion).*

Two groups of houses were selected in Okhla Village. One group was sprayed with turpentine emulsion and the other with the army emulsion, each being diluted so as to contain 1·25 per cent DDT. In each case the rate of spraying was 2 c.c. of emulsion, or 25 mg. of DDT per square foot of wall surface. One house in each group was left untreated as a comparison. During the first ten days the difference between the residual effect of the two emulsions was well marked. In the rooms treated with turpentine emulsion the reduction in the prevalence of female anopheline mosquitoes ranged from 82 to 89·4 per cent while in those treated with the army emulsion it ranged from 92·7 to 98·9 per cent. After the 10th day the residual effect of both emulsions fell rapidly. At the end of the second week the percentage of reduction was only about 50 per cent in both cases, and by the end of the 3rd week it had fallen to 33 per cent. When resprayed at the same dosage, the reduction of mosquitoes was maintained at about 82 to 84 per cent for about 5 weeks in the case of both the emulsions. It appears that such a small dosage is not enough to give reduction of mosquitoes for a prolonged period.

(b) *Turpentine-rosin-soap emulsion, MKE-rosin-soap emulsion and army emulsion.*

Selected portions of two separate villages have been sprayed with the above emulsion at the rate of 2 c.c. per square foot to determine their comparative efficacy and the duration of the residual effect. For one village all emulsions were diluted so as to contain 1·25 per cent DDT while for the other they contained 2·5 per cent. In the former village, in the portions sprayed with turpentine-toluene emulsion, the number of mosquitoes rose from 13 per cent on the 8th day after spraying to 76 per cent on the 23rd day. This area was resprayed on the 24th day. The number of mosquitoes after going down to zero per cent within 24 hours of spraying, rose to 45 per cent about 2 weeks after spraying. In the areas treated with the other two emulsions, the number of mosquitoes remained between zero and 42 per cent for about 8 weeks, the number in the part sprayed with the turpentine-toluene emulsion being comparatively lower than in the other portion.

In the other village in which 50 mg. DDT per sq. ft. were sprayed the number remained for about 9 weeks between zero and 6·5 per cent. As the weather became cold, this experiment was discontinued.

Observations indicate that the army emulsion is more effective than the MKE emulsion and the latter is more effective than the turpentine emulsion.

(c) *Army emulsion and DDT suspension in water.*

A village (Jogabai) was divided into three separate parts. One part (14 rooms—5,904 square feet wall surface) was sprayed with the army DDT emulsion, and the second part (11 rooms—5,156 square feet) was sprayed with DDT suspension in water, both sprays being diluted to contain 2·5 per cent DDT and applied at the rate of 2 c.c. of fluid or 50 mgs. of DDT per square foot, whilst the third was left untreated for comparison. Reduction in mosquito prevalence during the first five weeks has ranged between 82 and 99 per cent with the former and 94 and 100 per cent with the latter. For about 6 weeks the effect of the 2 emulsions was about the same, reduction in number of mosquitoes being maintained at about 100 to 80 per cent. During the sixth week, however,

the number of mosquitoes in the part sprayed with the toluene-turpentine emulsion increased to about 30 per cent and later came down again, while in the part sprayed with the suspension, the number remained down from zero to 20 per cent for nearly 89 days, after which the experiment was discontinued.

Another village (Juliana) was similarly treated, except that the spray was diluted to contain only 1·25 per cent DDT, only 25 mg. of DDT per square foot was applied. The observation made indicate that the reduction in the prevalence of female anopheline mosquitoes is higher in the rooms treated with suspension than in those treated with emulsion. As observed in the other villages in which 1·25 per cent army emulsion had been applied, the residual effect of this emulsion is high during the first ten days, but after that period it undergoes a rapid reduction.

(d) *DDT-turpentine-soap emulsion.*

When the walls of a verandah were being treated with a 5 per cent DDT-turpentine emulsion at the rate of 2 c.c. per square foot some potted plants (Dracena) were also sprayed along with the walls. Nearly two months later, female anopheline mosquitoes were allowed to remain in contact with the sprayed leaves for varying lengths of time, then removed and kept under observation in cages for 24 hours. The results of these observations are as follows :—

(i) DDT deposit on the leaves was effective even after 2 months.

(ii) The percentage of mortality among the mosquitoes after 24 hours was directly proportionate to the duration of contact with the leaves.

(iii) Sixty days after application, 14 minutes contact produced 84 per cent mortality after 24 hours and 98 per cent if the duration of contact was 20 minutes.

(e) *DDT and gammexane solutions in kerosene oil.*

A village (Massigarh) was divided into three parts. One part (22 rooms—9,635 square feet wall surface) was sprayed with 0·5 per cent solution of gammexane, the other (30 rooms—15,055 square feet wall surface) with 2·5 per cent solution of DDT (Commercial). In each case the solvent was kerosene and the rate of application 2 c.c. per square foot. In the rooms treated with gammexane the reduction in the prevalence of anopheline mosquitoes during the first week varied between 82 and 100 per cent but very little effect was apparent 8 days after the application of the spray. In the case of DDT solution, on the other hand, there has been a 90 to 100 per cent reduction for nearly three weeks.

A stronger solution of gammexane (containing 1 per cent gamma-isomer) was then applied. The observations in this section of the village show that the number of mosquitoes came done to zero within 24 hours and remained there for about 10 days, after which the mosquitoes began to increase in number and reached the original number on about the 13th day. A suspension of 666 in water containing 0·13 per cent and 0·25 per cent gamma-isomer was then sprayed. The number of mosquitoes came down to zero within 24 hours and remained below 15 per cent for over 4 weeks after which, due to cold weather, the experiment was discontinued.

(II) Quetta.

The following trials were undertaken at Quetta, the same procedure as at Delhi being followed :—

(a) Village Shadi Khan was treated indoors with a 5 per cent. solution of DDT in kerosene at the rate of 1 c.c. (50 mg. of DDT) per square foot.

(b) Village Rajab Khan was divided into two parts, one treated with turpentine emulsion, the other with toluene-turpentine emulsion, each diluted to contain 1·25 per cent DDT and sprayed indoors at the rate of 2 c.c. (25 mg. of DDT) per square foot.

(c) Village Subzal Khan was also divided into two parts, one part (much larger than the other) being treated indoors with 1·25 per cent supsension of DDT in water at the rate of 2 c.c. per square foot, the other being treated outdoors only with the same suspension, but at the rate of 4 c.c. per square foot.

Another small village (Kili Abdulla Jan) was left untreated as a comparison.

An analysis of the observations made in the different villages show that :—

(i) In the village sprayed indoors with DDT-kerosene solution, mosquitoes prevailed in insignificant numbers throughout the period of observation—up to nearly 7 weeks after treatment.

(ii) In the part of the village treated with the army emulsion, catches were continuously low after the first treatment. In the portion treated with turpentine emulsion the catches were not markedly reduced after the first treatment but fell precipitously after the second. This difference in the efficacy of DDT turpentine emulsion from that observed in the villages near Delhi may be due to the inferior quality of turpentine used at Quetta, but this question requires further investigation. In villages treated with suspensions catches in the section treated indoors were extremely low, but in the part treated outdoors only the mosquito population showed no reduction after the first application though it was somewhat reduced after the second treatment.

An analysis of the infection rates in mosquitoes has shown that infected mosquitoes are mainly found in either the untreated villages or in that portion of the village which was sprayed outdoors only.

(III) Conclusions.

From the field experiments conducted at Delhi and Quetta it is concluded that :—

(1) Indoor residual spraying, is the most effective method. Outdoor residual spraying is not effective either against *culicifacies* at Delhi or against *superpictus* at Quetta.

(2) For residual spraying, emulsions (oil in water) and suspensions (in water) containing 2·5 per cent DDT and applied at the rate of 2 c.c. per square foot are highly effective. One application in the Quetta (*superpictus*) area and two or possibly three, in the Delhi (*culicifacies*) area are probably sufficient to control the incidence of malaria during the whole malaria season.

6. Mammalian malaria enquiry under the Director, Central Research Institute, Kasauli.

Introduction.

The progress of the work of the mammalian malaria enquiry was restricted by the absence during the year of Lieut.-Colonel H. W. Mulligan and Major T. Sommerville, the former for 7 months on duty and leave and the latter for 6 months on leave. These officers had completed continuous spells of duty abroad for 8 and 11 years respectively and as it was decided to continue the enquiry until late in 1947 it was considered advisable that their leave should not be postponed. Partly for this reason and partly because a large quantity of material had already been collected for histological examination, the mosquito transmission section of the enquiry at Karnal was closed down in June without prejudice to its resumption in the following year should this prove to be necessary.

The Enquiry has now been in progress for 18 months but up to the present no evidence has been obtained that, in mammalian malaria an exoerythrocytic cycle occurs comparable to that which has been described in either Avian or Saurian malaria. All observations have so far been confined to infections with *P. cynomolgi* in *M. mulatta* (*rhesus*) monkeys. A brief review of the work so far carried out is given below.

Experimental mosquito transmission of *Plasmodium cynomolgi* to *Macaca mulatta* monkeys.

The experimental mosquito transmission of *P. cynomolgi* to *M. mulatta* monkeys was carried out in a large number of animals during the spring. Compared with the experience of the previous autumn the mosquito mortality was lower and the intensity of the infection in the mosquitoes higher, thus enabling a large sporozoite dosage to be given.

Successful transmission to all anopheline species which have been used in the experimental work has been obtained. The development of *P. cynomolgi* up to the formation of sporozoites in the salivary glands has been observed in *A. subpictus*, *A. annularis*, *A. culicifacies*, *A. barbirostris* and *A. hyrcanus*. Attempts to transmit the infections to culicines on the other hand have failed in every case. *A. annularis* was chiefly used in the spring as, owing to its prevalence, large numbers were readily obtained.

Experience showed that the effective working limits of temperature for the production of infective sporozoites lie between about 72°F. and 82°F. Below 72°F. the sporogony cycle is slow and the sporozoites produced are not usually infective. Above 82°F. the mosquito mortality is so high that only a few infected mosquitoes can be obtained. Studies were made on the relationship between the size of the sporozoite dose and the development of the infection in monkeys. The results indicated, however, that the quality of the sporozoites at the time of transmission was more important than their number.

Course of *P. cynomolgi* infection in *M. mulatta* monkeys.

A study of the course of *P. cynomolgi* infection in *M. mulatta* monkeys was carried out covering all stages of the infection. Certain interesting features are recorded below:—

1. *Incubation period.*

The average incubation period in sporozoite infected monkeys is 15 days.

The occurrence of a delay in the onset of the primary attack was observed in two cases. The exact duration of the incubation period is not known as, in both cases, the patent infection followed splenectomy.

In the first case the infection was introduced by means of an intravenous injection with sporozoites. No patent infection was detected 7 weeks after sporozoite inoculation, so a splenectomy was performed. The spleen was of normal size and had a normal appearance. The primary attack developed 52 days after splenectomy and 108 days after sporozoite inoculation. It lasted 8 days and the maximum parasite prevalence was 840 per 10,000 red cells.

In the second case the infection was produced by the bites of 11 infected *A. subpictus* mosquitoes. No patent infection had developed 5 weeks later so a splenectomy was performed. The spleen was of normal size and had a normal appearance. The primary attack developed 12 days after splenectomy and 50 days after exposure to the bites of infected mosquitoes. The attack lasted 14 days and the maximum parasite prevalence was 470 per 10,000 red cells.

2. *Stage of parasitæmia.*

The logarithmic phase of parasite development continues more or less unchecked until just before the peak of parasitæmia. From the ascending curve it can be calculated that all of the merozoites (average number 16) produced from one parasite at each cycle rarely infect new red cells, but that the number which do so averages 9·2 (S.D.=4·64). The peak figure varies greatly but is rarely more than 2,000 parasites per 10,000 red blood corpuscles. The descent from the peak is often prolonged and irregular, while traces of parasites may remain in the blood stream for weeks afterwards. Within the observation periods which have so far elapsed (up to 15 months in some cases) true relapses have occurred in only 2 out of 90 monkeys. In both cases the relapses were milder than the primary attacks.

Experiments on the transmission of *P. cynomolgi* by blood and tissue subinoculation during the incubation period following sporozoite injection.

A series of studies designed to determine the infectivity of the blood and tissues during the incubation period of *P. cynomolgi* infections in *M. mulatta* monkeys infected by sporozoite inoculation were carried out. The method employed to determine the infectivity of any particular tissue consisted in attempting to transmit the infection from an infected donor monkey to a recipient monkey by sub-inoculation of that tissue.

(a) *Blood sub-inoculation experiments.*

The quantity of blood used for sub-inoculation was usually about 15 c.c., and it was obtained from the heart of the donor monkey. This method was adopted for the collection of blood to obviate the difficulties which were experienced in collecting this amount from the saphenous vein. The intraperitoneal route was used for sub-inoculation into the recipient monkey.

The results of the experiments of the infectivity of the blood *immediately* following sporozoite injection were all negative. The intervals after sporozoite

injection at which blood sub-inoculation was carried out were 5 minutes, 10 minutes, 25 minutes, 37 minutes and 72 minutes.

In the experiments in which blood sub-inoculation was carried out at daily intervals after sporozoite injection successful transmission was not obtained until 9 days after the sporozoite inoculation. It was observed that successful transmission by blood subinoculation occurred 2 or 3 days before parasites could be detected in thick blood films.

(b) *Tissue sub-inoculation experiments.*

In the studies on the infectivity of the spleen during the incubation period two methods of subinoculation were employed. The first consisted in splenectomizing the donor monkey and transplanting the whole or a portion of spleen into the peritoneal cavity of the recipient monkey. The second method consisted in the intraperitoneal injection of spleen tissue which had been ground up in saline after removal from the donor. In each experiment a control was carried out consisting of the sub-inoculation of blood removed immediately prior to the splenectomy on the donor monkey.

The results of these experiments demonstrated that in no case was the spleen infective during the incubation period following sporozoite injection.

Similar experiments were conducted with other tissues but the method employed in these cases consisted in preparing a suspension of the organ by grinding up a portion in saline and injecting it intraperitoneally into the recipient. The tissues which were investigated included liver, bone-marrow, brain, lung, kidney, suprarenal, pituitary and muscle.

The results obtained were similar to those observed with spleen subinoculation in that none of the organs were found to be infective during the incubation period following sporozoite injection.

Recipient monkeys which came to post-mortem six months later showed no trace of the inoculum in the peritoneal cavity, the whole had been observed.

Conclusion :—No evidence could be found that the blood was infective at any time within the first nine days after sporozoite inoculation. The blood was infective after nine days. The tissues are not infective after sporozoite inoculation until the blood also is infective.

Localisation of sporozoites in the skin and spleen.

1. *Skin.*

Infected mosquito thoraces were ground up in monkey serum-saline mixture and injected into the skin of 14 monkeys in doses of from 6 to 104 thoraces. The inoculum was excised with a piece of skin after varying intervals from a quarter of an hour to four days. Control inoculations were made in six cases with uninfected material. The material was examined histologically.

In only one or two sections were sporozoites detectable, and these were biopsies made one hour after inoculation. Even so, only very few sporozoites were seen, though from the dose given it was calculated that one high power field of inoculum might be expected to show over 1,000 sporozoites. Not all the inocula were infective, however, only 39% of the animals developing infection.

In both the control and infected material the liquid part of the inoculum is seen to spread rapidly laterally along the tissue planes below the dermis leaving

the solid part (consisting chiefly of chitin and muscle) comparatively dry. In about a quarter of an hour vasodilatation is pronounced and soon after migration of neutrophil leucocytes takes place into the inoculum. In 8 hours the abscess is well established, and the undifferentiated mesenchymal cells in the subcutis are undergoing hypertrophy. These are playing a maximal part by about 48 hours, and form foam cells and histiocytes containing all manner of chromatophilic debris. It is in this phase that a difference was noticed between the histiocytes of one or two of the infected cases and those of the controls.

In these one or two cases the histiocytes were close to the abscess and swollen and ovoid in form. They contain bits of neutrophils and lymphocytes and also many dusky-pink globular bodies averaging about 4 microns in diameter. These bodies do not occur in any other cases, but one must bear in mind the fact that in no other case was the inflammatory reaction so marked. Their lack of organised structure makes it unlikely that these bodies are exo-erythrocytic forms.

One other noteworthy observation was in the behaviour of melanin. This substance occurs in two forms: (1) monkey melanin, in the resting histiocytes of the dermis and (2) mosquito melanin, not only in the chitin but in small brown-blue granules in the liquid part of the inoculum. The monkey melanophores hypertrophy as part of the inflammatory reaction, while the mosquito melanin granules get taken up by histiocytes and form cytoplasmic inclusions. As the monkey melanin is usually of a greenish blue, it can be distinguished from the mosquito melanin in histiocytes. The appearances presented simulate possible exoerythrocytic forms of the parasite and care must be taken to recognise their true character.

2. *Spleen.*

Similar experiments were carried out at the spleen. This organ was implanted in the abdominal wall so that biopsies might be taken at intervals after inoculation. In all, 32 biopsies were made on 10 inoculated spleens while 6 biopsies were made on 2 controls. Fifty per cent of the infected animals failed to develop malaria.

The course of the inflammation is similar to that seen in the skin, but necrosis, secondary infection and the appearance of giant cell macrophages is much more marked. In one or two cases, at 48 hours, there were numerous histiocytes containing blue granules to be seen near the inoculum, but the same reasons exist as in the case of the skin for ruling these out as exo-erythrocytic forms. Moreover the same variability in the intensity of the inflammatory reaction was to be observed.

Histological examination of tissues.

In addition to the biopsies already mentioned post-mortems have been made on 136 monkeys. A large collection of tissues has thus been made, which will serve as a research reference stock, if anything is discovered in the most promising material. The tissues of representative cases in all stages of the disease have already been examined without revealing any definite evidence of exo-erythrocytic forms comparable to those described in avian malaria.

1. *Technique.*

Films are stained by buffered Giemsa, while tissues are fixed in either formal saline or Zenker formol and are embedded in paraffin. Sections are

stained by a modification of Barrett's method, which proves satisfactory both for the cytology and for the parasites in their erythrocytic phase, and in the case of *P. gallinaceum* in their exo-erythrocytic phase also. Formol and malarial pigment can be conveniently removed by saturated picric acid in alcohol.

2. *Regional concentration of parasites.*

In the case of one monkey which was sacrificed at, or about, the peak of parasitæmia, a regional concentration of parasites was observed in the Billroth cords of the spleen. In arterial, venous and capillary blood only some 13% of the red blood cells were parasitized whereas the red cells of the Billroth cords were virtually 100% parasitized. The picture presented corresponds closely to that described and figured by Taliaferro and Cannon (1936) in the spleen of a monkey infected with *P. brasilianum* which had been sacrificed at the peak of parasitæmia. In our case there was no corresponding regional concentration of parasites in the lymph nodes.

Puzzles and fallacies encountered in the search for exo-erythrocytic forms of *P. cynomolgi*.

Numerous puzzles and fallacies have been encountered during the search for exo-erythrocytic forms of *P. cynomolgi* in the films and sections obtained from infected *M. mulatta* monkeys. Studies on the classification of these puzzles and fallacies have been undertaken and are in progress.

One interesting fallacy is an adventitial histiocyte which is found in the smallest vacules of the brain in all cases including the controls. It is an elongated cell which lies in rows or singly along the vessels which have practically no muscularis. It contains numbers of coarse and fine granules which stain a delicate blue with Giemsa in films and a rather greenish blue in sections. The granules are not metachromatic. They resemble the exo-erythrocytic forms which occur in the brain in avian malaria but they lack the organic structure of the exo-erythrocytic parasites.

The characteristic features of the majority of the fallacies encountered are their relatively infrequent occurrence, their lack of organized structure, and the fact that they do not resemble schizonts. In the majority of cases they occur in macrophages and are probably products of phagocytosis. In sections nuclei undergoing nitosis sometimes resemble exo-erythrocytic schizonts of avain malaria.

During the preparation of this report some interesting "bodies" were found in a section of psoas muscle obtained from a monkey which was sacrificed at the peak of parasitaemia. This monkey was infected by the bites of about 300 heavily infected mosquitoes, and when sacrificed showed a heavy infection with erythrocytic forms of *P. cynomolgi*.

The "bodies" observed were present in considerable numbers and had the appearance of cysts. They appeared to be located in the muscle fibre, measured approximately 40 μ in diameter, and showed a well defined cyst wall. They contained a large number of small structures which stained blue with Barrett's stain. The exact nature of these "bodies" is not at present understood, but nevertheless they represent the most promising lead since the enquiry started. Further work is in progress to determine the true nature of these cyst-like bodies and whether or not they are connected with the malaria infection.

Tissue culture.

By a simple technique, modified from that used by Hawking for *P. gallinaceum* in fowl spleen cultures, it is easy to grow histiocytes, fibroblasts and lymphocytes from monkey spleen. Cultures of the liver, however, yield only a very few of these cells; while those of the brain shown death of the capillary endothelium and growth of histiocytes, fibroblasts and lymphocytes from the adventitial cells and from the pia-mater. No growth of parasites has been obtained.

Estimation of the age of monkeys.

There is now abundant evidence in the case of birds that exo-erythrocytic forms grow more easily in young birds than in old ones and more easily in embryos than in either. There is slight evidence that *P. cynomolgi* infection is more severe in younger than in older monkeys. It is therefore of importance to know the age of a monkey. All the specimens of *Macaca mulatta* used in this enquiry are caught wild. Criteria of age can most conveniently be derived from study of the weight, sitting height and dentition of monkeys. Very useful tables of these have been published by A. Schultz in Hartman and Straus's book on the Rhesus monkeys. The author, however, does not indicate the variation by means of a standard error. It is, therefore, not possible to determine the error of applying their tables to individual wild caught monkeys. Our study of the height-weight ratios of 46 monkeys of "good" nutrition shows their growth curves to be the same as those found by Schultz, namely $(\textit{Sitting height})^3 = 18{\cdot}1$. It is expected that further study may yield useful information about the variation in the monthly or quarterly rate of growth of individual monkeys.

OTHER INVESTIGATIONS.

Tissues from Malabar squirrels infected with *P. ratufae* have been searched for evidence of exo-erythrocytic schizogony but without success.

7. Enquiry on adult *Anopheles stephensi* under the Director, School of Tropical Medicine, Calcutta.

1. *Daytime resting places :*—The daytime resting places of adult *Anopheles stephensi* in Calcutta had hitherto remained obscure, except for the basement of a derelict, unfinished building reported by Siddons (*Jour. Mal. Inst. Ind.*, Vol. VI, No. 4). A number of new daytime shelters were found during the present investigation; these include cowsheds (particularly one very dark stone-built shed), outhouses such as garages and godowns, and a group of combined human and animal (goat) dwellings. In addition it was established that the house which in previous years yielded *A. stephensi* in early morning catches is, in fact, a daytime shelter. Cattle were not observed in the vicinity of the basement, but the house and the outhouses from which catches were made were under the influence of bovine attraction. The number of adults caught in the different types of shelter were as follows: basement, 400 females and 124 males; cowsheds, 568 females and 2 males; house, 648 females and 74 males; outhouses, 158 females and 15 males; combined human and animal dwellings, 19 females. These figures have no comparative value in respect of

the different shelter as the latter have not been visited to the same extent. The species appears to rest indiscriminately in shelters of different type; sufficiently dark and accessible places close to the source of blood are favoured.

2. *Races*:—Samples of 10 ova from 221 batches were measured in order to obtain var. *mysorensis* which has been stated by other workers to be present in the city and which may be a vector here, because the race of some naturally infected specimens captured during the infectivity survey of 1943-45 was in doubt. 200 batches were of type form. 200 batches were immediate in respect of length, breadth or float length. It was found in several of these latter batches, that an increase in size had occurred when the eggs were re-measured about 20 hours later; the definitive measurements indicated type form ova. In other batches in which no significant increase was noted on re-measurement, in-breeding, in some cases to the F4 generation, has failed to recover true var. *mysorensis*. One batch of eggs was type form in most characters, but had an average ridge count of 14·6, rather suggestive of var. *mysorensis*. It has so far been inbred to the F3 generation and has yielded pure type form and ova like the F1 with a low ridge count. Measurements of retained eggs obtained by dissection and eggs of the same batch deposited in the natural way and measured after the chorion has hardened show that race determination cannot be done on the former type of material. No biological evidence of true var. *mysorensis* was obtained.

3. *Feeding habits*:—Over 600 blood meals have been collected from mosquitoes captured in different habitats towards a full year's study of the feeding habits of *A. stephensi* by means of the precipitin test.

4. *Seasonal prevalence, density, etc.*:—These subjects were being studied on the basis of the average catch, per period at three selected routine catching stations. After being in progress for three months, the work was interrupted by the dislocation of the routine catches due to disturbed conditions in Calcutta. The composition of the catches in respect of wing and abdominal condition is also being studied. The large amount of data collected is being analysed.

5. *Infectivity*:—250 guts and 1,093 glands examined during the last nine months of the year, which covers the transmission season, were negative for oocysts and sporozoites respectively. This is attributed to low density in combination with a probably low anthropophilic index.

6. *Experiments with marked mosquitoes*:—1,143 female and 935 male *A. stephensi*, all laboratory-bred under very favourable conditions (judging by period of development and size criteria) were marked with wet stains and released in the basement in 6 lots between 19-5-46 and 5-7-46. The total number of captures—3 females and 1 male—was most disappointing and did not come up to expectations on the preliminary trials with specimens marked with coloured powders. Controls kept in the laboratory lived long and showed the stains on test. The explanation is believed to lie in a change in the character of the immediate environment of the release point, a change which was not realised until some time afterwards. It was that whereas formerly a number of cows, both privately-owned and stray, were always to be seen there, this year cows have never once been observed there either at the time of the routine visits or on special night visits. If this is the correct explanation, it suggests a wider range of dispersal in the absence of the preferred host. No further experiments will be conducted until a suitable station can be found.

7. A number of specimens have been found parasitized by *Culicoides anopheles*, possibly a first record for *A. stephensi*.

8. Enquiry on the control of sandflies *Phlebotomus argentipes* by spraying with D.D.T. under Dr. D. N. Roy at the School of Tropical Medicine, Calcutta.

The work was started on the 1st September, 1946. For many reasons such as, unsettled conditions prevailing in Calcutta during August, September and October, 1946, and delay in obtaining staff, progress has been slow. According to the plan it was proposed to work out during this year, the seasonal variation in the prevalence of sandflies in all cowsheds and dwelling houses in five adjacent villages, Kaurapukur, Zaidargot, Rammakhal Chawk, Raghabpur and Thakurani Chawk and also to detect the source of their origin. The number of houses which have been periodically examined for sandflies in the different villages is given below :—

	Cowshed.	Dwelling house.
Kaurapukur ..	20	25
Zaidargot ..	20	25
Rammakhal Chawk	10	6
Raghabpur ..	10	6
Thakurani Chawk ..	10	12

Only 3 (three) species of sandflies have been encountered ; among them *argentipes* was the most numerous.

The question as to what extent sandflies are capable of breeding in human dwelling houses and to bite man are receiving very close attention. Till the end of December 1946; 463 examples of stomach contents of sandflies comprising *argentipes* and *papatasi*, have been subjected to precipitin tests against human and cattle.

Sugar has now been made available and the investigation into the breeding of sandflies will commence.

NUTRITION.

9. Nutrition researches under Dr. V. N. Patwardhan at the I.R.F.A. Nutrition Research Laboratories, Coonoor.

(I) Studies on vitamins of the B complex.

(a) *Interrelationships of the vitamin B group :*—The interrelation between the vitamins of the B group in rats in thiamin, riboflavin and biotin deficiencies has been studied. The riboflavin concentration in the liver was found to increase in thiamin deficient rats and in riboflavin deficient rats there was an increase in liver-thiamin. In biotin deficiency, the riboflavin in the liver increased while thiamin showed a decrease. The activities of the aldehyde oxidase, lactic and succinic dehydrogenases in thiamin, riboflavin and biotin deficiency in rats have been studied. These have been shown to be dependent on the relative concentration of thiamin, riboflavin, nicotinic acid and biotin in the tissues The oxidation of aldehyde and pyruvic acid appears to involve all these vitamins, thiamin, riboflavin and biotin, since withdrawal of any one of these appears to decrease the activities of their respective enzymes.

(b) *The estimation of thiamin by the thiochrome method :*—Several substances have been tested for their interference in the estimation of thiamin. Mercuric chloride, silver nitrate, iodine, bromine and potassium permanganate appear to interfere markedly ; hydroxylamine, hydrogen sulphide, sodium hydrosulphite and sodium pyrophosphate were found to be less interfering. The influence of the interfering substances was found to be a function of their relative concentration. pH of the reaction mixture within the limits investigated was not found to exert an inhibitory effect.

(c) *The estimation of para-amino benzoic acid :*—Experiments are in progress for the estimation of para-amino benzoic acid by using diazotized thiamin. The preliminary experiments indicate that the method is useful for estimating quantities above 20 micrograms. The diazotization of the para-amino benzoic acid followed by coupling with thiamin is also being investigated for developing a method for the estimation of the latter. One advantage of this method, if successfully developed, will be the elimination of the fluorimeter, an essential equipment for the existing methods.

(II) Lathyrism.

The effect of feeding khesari gram (*Lathyrus sativus*) to guinea-pigs in diets containing adequate quantities of known vitamins have been studied and compared with the effects obtained by incorporation of comparable quantities of Bengal gram, green gram and black gram respectively in diets of similar composition. Guinea-pigs grew normally on the diets containing Bengal, green and black grams. When the khesari gram was included at a level higher than 50 per cent the guinea-pigs did not grow. The animals developed certain symptoms suggesting toxic effects due to feeding with khesari gram. They were : extreme reluctance to move about, alopecia, dermatitis and in certain cases trophic ulcers in the hind legs. There was no obvious paralysis of the hind legs. Young and adult, male and female guinea-pigs were equally affected. Feeding khesari gram at 30 per cent level together with 50 per cent of wheat flour did not result in the development of the above symptoms.

The harmful effects of khesari gram feeding could not be alleviated by the addition of vitamins A, C and B complex, hence it is suggested that they might be of toxic origin.

(III) SUPPLEMENTARY VALUE OF FOODSTUFFS INVESTIGATED WITH POOR RICE DIETS.

(a) *Groundnut and coconut oil cakes* :—Experiments using albino rats are in progress to evaluate the supplementary value of groundnut and coconut oil cakes to diets based either wholly on roots and tubers or partly root-tuber and partly cereal, simulating the present day conditions obtaining in some parts of South India.

On tapioca diets rats lost weight and four out of six animals died within six weeks. When 30% of tapioca was replaced by coconut cake the rats showed an average growth of 8 gms. per week.

(b) *Food yeast* :—The supplementary value of the food yeast to the poor rice diet has been tested on albino rats by the growth method. The supplement of food yeast was added at three levels : 0·5, 1·0 and 1·5 oz. respectively to 23 parts of the poor rice diet. The supplement of 0·5 oz. gave the best growth as compared with the higher levels of supplementation. It also compared favourably with the effect of feeding groundnuts 0·5 to 1·5 oz., red gram and black gram 1·5 to 3 oz. 1·5 oz. of skim milk powder supplement gave a much better growth as compared with the food yeast. The experiments show, however, that with other commonly obtainable substances as supplements mentioned above, the food yeast shows fairly good results.

(IV) CLINICAL INVESTIGATIONS.

Investigations on nutritional disorders are being carried out at the Nutrition Clinic and Research Ward, Stanley Hospital, Madras.

(a) *The clinical features of riboflavin deficiency* :—Contrary to the experience in other parts of India, it was found that the commonest signs of malnutrition in patients coming to the hospital at Madras, were those attributable to deficiencies of vitamins of the B complex. The clinical features of the riboflavin deficiency were found not only in the oro-lingual and ocular mucous membranes, but in other mucous membranes and mucocutaneous sites in the body as well. In women of child-bearing age, leucorrhea characterised by a thick viscid discharge, acid in reaction and rich in Doderlein's bacilli, was observed. In some cases this was associated with pruritus vulvæ. In children, vulvitis appeared as a manifestation of riboflavin deficiency. These conditions sometimes were found to abate under riboflavin therapy.

Some cases of anal fissure and proctitis were also found to improve under riboflavin treatment. Anal fissure, proctitis, angular blepharitis and preputial ulceration, are therefore considered other possible manifestations of riboflavin deficiency.

(b) *Ætiology of phrynoderma* :—The hypothesis that the disease was due to vitamin A deficiency had found general acceptance. But recent work has exposed several fallacies which would necessitate a close examination of the current hypothesis. In Stanley Hospital, Madras, a group of 116 cases of phrynoderma were investigated. There was gross lack of parallelism between

the incidence of phrynoderma and xerophthalmia. On the other hand there was a distinct correlation between the incidence of phrynoderma and the signs due to deficiency of the vitamins B complex. 37 of these cases were subjected to therapeutic tests. The cases were divided into six groups which were treated with vitamin A concentrate, nicotinic acid, riboflavin, yeast extract, linseed oil and yeast extract *plus* linseed oil, respectively, for periods ranging from five to six weeks. Treatment with yeast extract was successful, but the improvement was slow. Linseed oil by itself, however, could beneficially influence the cure of phrynoderma in many cases. The combination of yeast extract and linseed oil yielded highly satisfactory results, whereas the other treatments mentioned above were found to be unsatisfactory.

(c) *The "Burning Feet" Syndrome*:—A deficiency disease characterised by a burning sensation in the extremities found in association with symptoms of ariboflavinosis has been investigated. The symptoms were relieved by Marmite therapy and by injection of calcium pantothenate, but not influenced by treatment with thiamin, nicotinic acid and riboflavin.

(V) Analyses of foodstuffs.

(a) *The vitamin A content of fish liver oils and carotene content of some foodstuffs*:—The vitamin A content of shark liver oils obtained from several places in India has been determined by the tintometric method. The values were found to vary from 110 I.U. per g. of oil to 37,900 I.U. per g. Some oils were subjected to the determination of vitamin A by both the tintometric and the spectrographic method. The relation between the International Units of vitamin A per gram determined by spectrographic method and the Carr-Price value was found to vary considerably. The figures ranging from 43·6 to 64·2 were obtained during the course of investigations. The carotene content of about 23 different foodstuffs was also investigated.

(b) *Coconut*:—The kernel of the coconut has been analysed. Moisture 2·15%; crude protein 8·27%; ether extract 68·41%; ash 2·10%; carbohydrates by difference 19·07%. About 58 per cent of the total nitrogen in the kernel is water soluble and consists mainly of proteins and metaproteins. The essential amino-acid content of the proteins of coconut is being investigated. The coconut contains only 12% of its total phosphorus in the form of phytin phosphorus.

The coconut is poor in many vitamins which were tested for. Carotene and riboflavin are almost absent. Choline, pyridoxine, thiamin and nicotinic acid are present in small amounts.

(VI) Nutrition classes.

No nutrition classes were held in the year 1946.

(VII) Educational and advisory work.

Requests for information and advice were received from all over India including the Central and Provincial government departments, private individuals and firms. The Nutrition Museum in the Laboratories was visited by numerous people.

10. Ascorbic acid enquiry under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta.

The bio-synthesis of ascorbic acid by the rat.

Investigation on the problem of the bio-synthesis of ascorbic acid by the rat under normal or narcotised condition has been continued on the following lines :—

1. *Attempts at accelerating the in vivo synthesis of ascorbic acid by the rat flushed with thiamine and pyruvic acid :*—It has been previously reported that chloretone induces an acceleration of the normal synthesis of ascorbic acid by rats and this induced effect can almost completely be suppressed by making those animals deficient in thiamine. In addition, the narcotic has also been found to produce certain inhibition, as indicated by the methylene blue technique, of the dehydrogenase system responsible for pyruvic acid oxidation. It is also well known that thiamine is a primary factor for the oxidation of pyruvic acid in animals (Peters *et al.* Biochem. J. 1937, **31**, 1637 ; Barron *et al.* J. Biol. Chem. 1941, **141**, 957). It, therefore, appears that thiamine is essential both for the oxidation of pyruvic acid and the acceleration of ascorbic acid synthesis in the rat.

Attempts were, therefore, made for raising the thiamine and pyruvic acid levels in rats by flushing those animals with high doses of these substances at regular intervals and to study the effect on the synthesis and excretion of ascorbic acid by them. It was observed that an injection of additional thiamine and pyruvic acid into rats has no significant effect on the normal excretion or the tissue reserve of ascorbic acid of the animals.

2. *The effect of chloretone on the aerobic and anaerobic oxidation of pyruvic acid by brain tissue of the rat :*—The effect of chloretone on the oxidation of pyruvic acid by the brain tissue (minced) from rats under normal or narcotised* condition was studied with the manometric technique of Warburg. For the anaerobic oxidation the ferricyanide technique of Quastel *et al.* (Biochem. J. 1938, **32**, 936) was used which depends on the fact that in the reduction of 1 mol. of ferricyanide, 1 mol. acid is formed and in bicarbonate media this gives rise to 1 mol. of CO_2 which can be estimated manometrically. It was observed that the aerobic oxidation of pyruvic acid by the brain tissue from the rat was not significantly affected by the narcotised condition of the animals, but if a very small quantity of chloretone (0·2 ml. of a 0·5% solution) were added to the medium bathing the tissue material in the Warburg vessel, a definite inhibition (30-45%) of pyruvic acid oxidation was observed. Since nearly 24 hours elapsed between the experiments on the surviving tissue and the last administration of the narcotic to the animals for inducing enhanced ascorbic acid excretion, it is possible that much of the effect of the narcotic in the intact animals was lost by that time. So addition of a very small quantity of chloretone simply helped that inhibitory effect. If the rats were injected 2 or 3 times with chloretone (10 mg. at a time) about 15-30 minutes before the animals were sacrificed for experiments, a definite inhibition of the pyruvic acid oxidation was then observed. On the other hand the anaerobic oxidation of pyruvic acid by the rat brain was not affected by the narcotised condition of the animals or chloretone being added to the surviving tissue.

* 'Narcotised' stands for condition of the animal given chloretone daily at 20 mg. per 100 g. body-weight for at least 1-2 weeks, if not more.

It appears, as suggested by Michaelis *et al.* (Biochem. J. 1941, **35**, 519), that chloretone exerts its main effect on that part of the system responsible for the oxidation of pyruvic acid under aerobic condition and not on that component capable of oxidising it anaerobically. It has been previously reported by the workers on this enquiry from a study of different dehydrogenases present in the rat tissues, that succinate system is not affected by chloretone and Michaelis *et al.* (*ibid.*) have shown that the narcotic has little or no inhibitory effect on the activity of cytochrome oxidase. It appears from the results obtained by this enquiry and which has also been suggested by these authors that a flavoprotein forming an important link between the cozymase and cytochrome system, is the narcotic sensitive component in the process.

3. *The effect of riboflavin deficiency on the biosynthesis of ascorbic acid by the rat* :—Since it appears from results obtained by this enquiry that a flavoprotein is affected by the narcotic it was of great interest to investigate the result of riboflavin deficiency on the normal synthesis of vitamin C by the rat or its stimulation under chloretone.

It was observed that chloretone could not stimulate to any significant extent, the urinary excretion of vitamin C in riboflavin-deficient rats even with daily administration of the narcotic to those animals for 1-2 weeks, while with the controls, paired-fed, the usual onset of enhanced excretion began much earlier and reached the usual maximum (6-8 mg. per 24 hours) within a week or so. When the riboflavin-deficient-narcotised rats were subjected to B_2-therapy (1-2 mg. each) along with the daily dose of the narcotic, the urinary excretion of vitamin C by those deficient animals began to rise and reached the usual maximum within 2-3 weeks.

It was further observed that the tissue reserve of ascorbic acid is appreciably reduced in rats under riboflavin deficiency. This reduction in tissue content (nearly 50% in case of liver and kidney and 15% for brain) appeared to be not due to inanition or any secondary effect, resulting from a depletion of the vitamin in the animals. It seems, therefore, that the normal synthesis of ascorbic acid, as reflected in the tissue storage of rats or its stimulation under chloretone is dependent on riboflavin. It has been previously reported by this enquiry that these processes also require the intervention of thiamine. So both thiamine and riboflavin are essential for the normal synthesis of ascorbic acid by the rat or its stimulation under chloretone.

4. *The question of the normal synthesis of ascorbic acid by rats deficient in thiamine or riboflavin* :—A question naturally arises that, if the vitamin C synthesis by the rat be so absolutely dependent on thamine or riboflavin then how does the normal process proceeds at all and the tissue storage of ascorbic acid considerably maintained when the animals are made acutely deficient with respect to these vitamins (B_1, B_2). It has been observed by this enquiry that in rats which were nearly on the point of death due to acute polyneuritis, the brain and kidney tissues still retained an appreciable amount of thiamine though the liver was found to be very nearly depleted of this vitamin. Ochoa *et al.* (Biochem. J. 1939, **33**, 2008) have also shown that even in the extreme state of riboflavin deficiency the rats retain in their organs an appreciable amount of the nucleotide. The rats cannot, therefore, be completely depleted of thiamine or riboflavin. The synthesis of vitamin C by them, therefore, goes on, though at a diminished rate, with the help of the amounts of B_1 and B_2 still retained in the tissue of the deficient animals. It seems, however, that the stimulating

effect of the narcotic does not come into play without an appropriate tissue level of thiamine and riboflavin in those animals.

5. *Question of the inactivation of ascorbic acid oxidase by the narcotic :—* It has been observed that rat tissues, particularly brain, liver and kidney, do not contain any ascorbic acid oxidase. Moreover, the enzyme prepared according to Tauber *et al.* (J. Biol. Chem. 1935, **110,** 211) has been found to be unaffected by chloretone. The possibility of inactivation of the oxidase by the narcotic and thus sparing of the vitamin C from the destructive effect of the enzyme, leading to a greater urinary excretion by the rat must, therefore, be excluded. The inactivation idea is also highly improbable from other considerations. Nelson *et al.* (J. Am. Chem. Soc. 1940, **62,** 1409) have isolated crystalline ascorbic acid oxidase and found it to be copper-protein compound. Metal-protein oxidase, as a rule, are not narcotic sensitive (Elvehjem, Respiratory Enzymes, 1939).

It appears from the observations so far recorded that both ascorbic acid synthesis and pyruvic acid metabolism in rats required the intervention of thiamine and a flavoprotein. Both these processes are peculiarly affected by chloretone. It is, therefore, very likely, that the pyruvic acid metabolism and the synthesis of ascorbic acid, as occurring in rats are very closely-related phenomena. From that standpoint pyruvic acid as a precursor for the synthesis of vitamin C by the rat also appears probable.

The fundamental significance and implications of the present work are quite apparent from the results so far obtained. Besides throwing some new light on the vexed problem of the synthesis of ascorbic acid by animals, it has established a definite relationship bewteen thiamine and riboflavin on the one hand and ascorbic acid on the other. It has also opened up a possible line of approach to the industrial synthesis of vitamin C from pyruvic acid.

11. Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

Deprivation of protein leads to an early loss of tissue protein and plasma protein. Hypoproteinaemia is undoubtedly one of the most significant causes of nutritional oedema. Anaemia is one of the conditions to be anticipated when the protein supply of the body is reduced for one reason or another. The development of anaemia as a reduction of body protein can be anticipated when one considers that haemoglobin is 95 per cent protein substance. Clinical observations support the conjectural possibility of development of anaemia due to depletion of body protein.

Attention to nitrogen balance and the level of plasma protein has of late years come to be recognised as an important part of nutritional care of protein deficiency states. Nitrogen balance studies were carried out in 3 cases of nutritional oedema, 3 cases of anaemia associated with oedema and in 1 case of cirrhosis associated with malnutrition. Nitrogen balance studies were carried out for a varying periods of time throughout the course of recovery. The plasma protein values were determined periodically.

Blood counts—R.B.C., W.B.C., Hb content in g. per 100 c.c., colour index and cell volume were determined from time to time in all the cases of anaemia.

Selection of cases :—All the patients had oedema on admission. Clinically these patients were free from any cardiac or renal involvement. All showed symptoms of purely nutritional deficiency. They had no associated infection. The total protein was below 5·5 g. per 100 c.c. The values of globulin were within normal range. For comparison one patient was included in the series declared clinically as one of cirrhosis of liver.

Diet :—A large number of foodstuffs may be selected as a source of nitrogen. Milk is an excellent food although its water content is unnecessarily high. All the patients were kept on a high protein diet. Each patient was given 6 feeds each of 8 oz. of liquid. In addition they were given a concentrated protein diet containing high carbohydrate prepared by shaking 100 g. of milk powder in 200 c.c. of water. This gives 34 g. of protein and 52 g. of carbohydrate per feed. Two feeds of this preparation were given.

Results :—Increase in the plasma protein beyond a certain limit was found to be more difficult to demonstrate than the simple maintenance of a positive nitrogen balance. The case of cirrhosis also showed considerable clinical improvement on high protein diet. There was only one instance in which the protein value reached the normal level (case 1). The results of the plasma protein changes during the course of the studies are included in Table I.

TABLE I.

The changes in the levels of plasma protein in 3 patients of nutritional oedema and in 1 case of cirrhosis when kept on high protein diet.

No.	Total Protein.	Albumin.	Globulin g. per 100 c.c.	Eu-globulin.	Pseudo-globulin.	Fibrinogen.	Oedema.
A. *Cases of nutritional oedema.*							
Case 1 15-8-46	4·52	2.52	2·00	..	..	..	+++++
5-9-46	5·50	3·32	2·18	0·6	1·58	serum	+
2-10-46	7·12	4·22	2·90	0·94	1·62	0·34	No oedema.
Case 2 19-6-46	4·93	2·45	2·37	0·83	1·36	0·18	+++++
30-6-46	5·56	3·00	2·56	0·78	1·54	0·24	+
13-7-46	5·90	3·24	2·66	0·80	1·65	0·21	Nil
Case 3 9-8-46	3·43	1·00	2.43	0·90	1·32	0·21	++++++ +++
25-8-46	5·12	2·19	2.93	1·20	1·38	0·35	++
5-9-46	5·35	2·55	2.80	1·09	1·54	0·17	+
B. *Case of cirrhosis.*		*Case* 4.					
6-8-46	6·87	2·56	4.31	2·40	1·69	0·22	Acsites +++++
16-8-46	6·37	2·85	3.50	1·30	1·82	0·38	+++
12-9-46	6·25	3·13	3.12	1·21	1·72	0·19	++

Case 1. This patient showed considerable improvement with high protein diet with usual line of treatment. The oedema almost disappeared when the total was at 5·56 g. per 100 c.c. After 15 days almost normal value of total protein was reached.

Case 4. The patient with cirrhosis also showed considerable clinical improvement. The ascites showed a tendency to decrease. The protein values are also significant of improvement as shown by a tendency towards an increase in the value of albumin and a decrease in globulin due to a decrease in euglobulin.

Cases of anaemia :—All the cases of anaemia on admission had the values of R.B.C. below 2 million and Hb. below 4 g. per 100 c.c. These patients showed oedema at a higher level of protein. These patients showed better response to treatment with liver extract and a diet with high protein.

One patient developed oedema during recovery from anaemia. A female aged 36 had oedema with slight oedema at the ankles. The R.B.C. count was 1·05 millions, Hb. 4 g. per 100 c.c. Plasma protein 5·01 g. per 100 c.c. and 2·8 g. With liver extract she showed an improvement in the R.B.C. 2 million, Hb. 4·12 but developed massive oedema of feet, thigh, with ascites. There was a reduction in the value of total protein from 5·01 to 4·12 g. and a lowering of albumin from 2·8 g. to 2·12 g. per 100 c.c. Response to mercurial diuretic was not satisfactory. With double fortified plasma the ascites disappeared. She improved on high protein diet. There was an increase in the plasma protein values along with the increase in the red blood count and in Hb value. This case clearly indicates the extra demand of protein by the body during the regeneration of the R.B.C. during the course of treatment. In the absence of adequate protein there is a demand of tissue protein which leads to a depletion of plasma protein.

A more systematic work on the value of food protein in the regeneration of plasma protein in cases of nutritional deficiency states is in progress.

12. Nutrition research unit at Dacca University, Dacca.

Metabolism of fat.

The deleterious effect of coconut oil on the metabolism of calcium in human beings was reported last year and ascribed to the insolubility of the calcium-salts of some fatty acid constituent of the coconut oil.

The experiment was repeated with adult rats as experimental animals, with some modification. One diet supplied to the human subjects in the previous experiment was more or less typical poor Bengal-diet containing about six per cent protein of vegetable origin and a very small amount of calcium. The nutritional state of the subjects, so far as calcium is concerned, was also not good, most of the subjects being already in negative calcium balance and one just on the verge of it. The diet now used contained more than ten per cent protein (both of vegetable and animal origin) and a liberal supply of calcium in the form of salt-mixture. It is found that the excretion of calcium is much more with coconut oil than without it. The rats maintained a positive calcium balance but the retention of calcium decreased considerably with administration of coconut oil. This experiment, therefore, supports the observations made last year.

As reported earlier, attempts were being made to obtain the different fatty acids in sufficient amount from coconut oil. The purpose was to study the effects of each of these fatty acids on the calcium metabolism, for it was hoped that such study might throw some light on the deleterious effect of coconut oil. The said fatty acids have been obtained in sufficient amount and their effect on the retention of calcium is being studied.

Effect of different doses of nitrogenous manures (ammonium-sulphate and farm-yard manures) on the nutritive value of rice.

Analysis was undertaken of forty samples of the same strain of rice (Latisail, a transplanted aman) grown with different doses of nitrogenous manures such as ammonium-sulphate and farm-yard manure in the Government Agricultural Station, Dacca. The protein content of the samples presents a very significant result. With gradual rise of the amount of manures administered, the protein content of the rice also rises from 6 p.c. to 12 p.c. which is much higher than the usual value. It is definitely proved that administration of nitrogenous manures has got an important effect on the composition of rice.

The mineral composition and vitamin content of these rice samples are being estimated.

Vitamin B-complex.

Vitamin B_1 :—The effect of ingestion of protein at different levels on normal as well as polyneuritic albino rats, on the excretion of " Bisulphite Binding Substance " has been studied. It has been noted that when B_1-intake is inadequate—there is an increase of " Bilsulphite Binding Substances " in the urine. So the determination of B.B.S. in the urine provides an index for B_1 excretion. From the experimental results it is evident that protein ingestion reduces B.B.S. in the urine of normal as well as polyneuritic rats, indicating thereby that protein has got sparing action on vitamin B_1.

The effect of ingestion of fat at different levels on rats, kept on a B_1-free diet has also been studied. But there seems to be no regularity in the excretion of B.B.S. on the ingestion of fat at different levels.

The effect of quinine on rats kept on vitamin B_1-free diet is also being studied.

Nicotinic acid :—Attempts were made to find out if there is any relationship between carbohydrate metabolism and nicotinic acid. Previous investigations in this laboratory on this line were carried on rats. The present investigations are, however, being carried on rabbits and the latter are definitely more suitable and desirable for the purpose, the former being known to be able to synthesize nicotinic acid (or its amide) in their system.

Rabbits were administered nicotinic acid (10 mgms. or more) intravenously in normal saline solution and reducing sugar in the blood, before and after administration, was measured. The results obtained with rabbits were similar qualitatively to those obtained with rats. The glucose content of blood per 100 c.c. rose from normal level of 147 mgm. to 214 mgm. within half an hour of the administration and approximately maintained that value for more than an hour, and then fell slowly to the normal. Evidently some glucose or reducing substances of the kind have been mobilised from some source. The determination of the glycogen content of liver before and after the administration of nicotinic acid is in progress.

Soyabean.

(1) Growth experiment with soyabean milk :—Growth experiments on rats with soyabean milk and cow milk as supplements to a typical poor vegetarian rice diet revealed that the average increase in body-weight of rats receiving a supplement of soyabean milk was slightly lower than that with cow's milk supplement, but more than double that on rice diet alone. Estimation of the total mineral content of rice diet revealed that the amount of calcium with regard to phosphorus is extremely low and most unsuitable for the proper utilisation of these minerals. Soyabean milk and cow milk were also analysed for their calcium and phosphorus content. Calcium content of cow milk was found to be about four times that of soyabean milk. Another series of growth experiment on rats, in which soyabean milk was further supplemented with calcium in the form of lactate and gluconate so as to make the calcium and phosphorus content equivalent to that of cow milk, have been carried out. This further supplementation of soyabean milk has been found to improve the growth of rats and calcium gluconate has been found to be a better supplement than calcium lactate. But even after this fortification, soyabean milk is found to be slightly inferior to cow milk in promoting growth of young rats.

TABLE I.

Growth experiment with soyabean milk fortified with calcium.

Diet.	Average increase in body-weight of rats per week.
Poor rice diet	3·55 gm.
Poor rice diet with cow milk (10 c.c./day)	8·83 gm.
Poor rice diet with soya milk (10 c.c. of sp. gr. 1·031/day)	7·28 gm.
Poor rice diet with soya milk fortified with calcium gluconate (10 c.c. of sp. gr. 1·031/day)	7·75 gm.
Poor rice diet with soya milk fortified with calcium-lactate (10 c.c. of sp. gr. 1.031/day)	7·68 gm.

Further experiments on biological value of soyabean milk protein by balance-sheet method and growth method are in progress.

(2) Removal of unpleasant odour and taste from soyabean milk : Soyabean milk prepared in the ordinary way has got some unpleasant odour and a bitter taste and is also found to be less stable. These are mostly due to some unsaturated lower fatty acids present in soyabean. To get rid of those disadvantages, soyabean was subjected to different methods of processing before the preparation of milk. These treatments consists of soaking for certain hours, steaming, heating with different solutions and thorough washing. Milks prepared from soyabean after these treatments were found to be quite palatable and more digestible. No appreciable change of composition of milk was found to occur by these pre-treatments.

(3) Growth experiments with soyabean and other pulses at a lower food intake :—Although soyabean contains more protein, fat, and vitamins, it is not superior to other pulses in promoting growth of young rats when kept on diet fed *ad libitum*. To see whether soyabean has got supplementing effect superior to that of Bengal gram, on poor rice diet at a lower food-intake, a growth experiment was performed on a group of young rats.

TABLE II.

Growth experiment at a lower food intake.

Diet.	Average increase in body-weight of rats per week. At lower food-intake.	At food-intake *ad libitum.*
Poor rice diet	2·82 gm.	3·45 gm.
Poor rice diet with soyabean	3·52 gm.	5·86 gm.
Poor rice diet with Bengal gram	3·48 gm.	6·02 gm.

Further investigations are in progress.

Lathyrism.

Twelve pigeons (young) from same colony and almost of the same weight were chosen for the experiment. The animals were divided into three groups. The first group (A) was fed with normal diet noted below, the second group (B) was fed with experimental diet and vitamin A in the form of shark-liver oil, and the third group (C) was kept on the experimental diet only. The object of adding vitamin A with the diet of group (B) is to find out whether vitamin A has some direct bearing on lathyrism as reported by some workers.

The composition of the two diets is given below :—

Normal diet :—	Per cent.	Experimental diet :—	Per cent.
Ground gram	.. 20	*Lathyrus sativus* (Teori)	.. 31
Skimmed milk powder	.. 7	Starch ..	.. 58
Food yeast	.. 2	Food yeast ..	.. 2
Sodium chloride	.. 1	Calcium carbonate	.. 1
Calcium carbonate	.. 1	Sodium chloride	.. 1
Whole meal flour	.. 69	Skimmed milk powder	.. 7

N.B.—The nitrogen content of the two diets has been made approximately equal.

The animals on diet containing *Lathyrus sativus* alone (group B) and with vitamin A (group CO) showed retardation of growth at the end of seven weeks while those on normal diet (group A) showed normal progress in growth. The animals in group B and in group C seem to show a distinct tendency towards the development of lathyrism but the administration of vitamin A mitigates to a certain extent the development of lathyrism induced by *Lathyrus sativus*. Histological investigation on the different organs of both the experimental and normal animals are in progress.

Publications.

A review of the work done during the last eight years in this laboratory on metabolism of protein, fats, and minerals on human subjects has been published, as a special I.R.F.A. report. Work done on soyabean has also been published as a technical report jointly with Coonoor, Bombay and Lahore workers.

13. Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay.

(1) Fat metabolism.

The investigations reported last year under this heading have shown two important findings. Firstly, it is suggested that the rate of absorption of fat from the intestine is determined among other things by the presence of fatty acids of low molecular weight constituting the fat, while the change in saturation of the acids has no appreciable effect on the rate of absorption. Secondly, on a low intake of fat (0·80 per cent). There was a reproductive failure in rats in addition to the retarded rate of growth.

The experiments are therefore undertaken to further elucidate these points. It is intended to correlate the rate of absorption of fats with other physical and chemical constants of the fats and fatty acids, such as refractive index, surface tension, viscosity, mean molecular weight and melting and solidifying points. These constants are being determined at 37·5°C. so as to get data more comparable with the physiological process. The reproductive failure was thought to be probably due to lack of essential fatty acids and certain vitamins of B-group. Work is in progress to throw more light on this aspect of the fat metabolism.

(2) Nutritive value of milk and curds.

The work on this problem is further continued by using pure strains of acid-forming bacteria for the curdling of milk and noting its effect on the vitamins of B-group. It is also intended to extend the study to note the effect on other nutritional factors, such as proteins, fats and carbohydrates.

(3) Vitamin contents of vegetables.

Ten varieties of locally consumed leafy vegetables have been subjected to analyses for their carotene, nicotinic acid, and ascorbic acid contents. The following table shows the mean results of five determinations on each vegetable.

Table I.

The vitamin contents of leafy vegetables.

(Mean of five samples)

	Local name.	Water per cent.	Nicotinic acid.	Carotene.	Ascorbic acid.
		gms.	mgm. p.c.	mgm. p.c.	mgm. p.c.
1.	Alu (leaves) ..	82·1	0·884	5·20	9·05
2.	Alu (stem)	93·9	1·240	3·75	7·09
3.	Chuka	93·2	0·940	4·12	7·68
4.	Ambadi (white) ..	91·4	0·802	5·40	4·62
5.	Math (white)	90·2	0·800	5·12	3·85
6.	Tandulja	88·9	1·480	6·52	3·62
7.	Pokla (red)	89·5	1·010	8·48	10·98
8.	Rajgira	88·9	1·400	5·78	4·00
9.	Chavali	90·3	0·902	5·12	7·38
10.	Methi (small)	94·9	0·804	2·72	4·23
11.	Math (red)	89·2	0·725	9·96	5·50

The estimations of other vitamins as well as the inclusion of more local vegetables in the study is contemplated.

(4) NUTRITIVE VALUE OF PURE BRED STRAINS OF CEREALS AND PULSES.

An important step in the direction of collaborations between the agriculture and nutrition science in India was taken by this unit when it was decided to study the effect of strain and environmental conditions on the nutritive value of some cereals and pulses. The reports of similar investigations in Western countries and the U.S.A. are encouraging and hence reviewed from time to time in 'Nutrition Reviews'.

Fiftyfive samples of different strains of rice, wheat and pulses from various agricultural experimental stations in the Presidency have been analysed for their proximate principles, ash and mineral contents. The vitamins such as nicotinic acid, and carotene and essential amino acids such as methionine and cystine have also been estimated. The results have shown that the vitamin content is greatly influenced by the genetic nature of the strain ; the effect of other environmental conditions, however, appears to be of a minor character. This study emphasises the need for the development of new strains of cereals and pulses as a step forward towards the development of nutrition of this country. It is suggested that researches on this line by I.R.F.A. Nutrition Research Units in collaboration with the Provincial Agriculture Departments would yield fruitful results. The results of this investigation are under publication.

(5) A SEARCH FOR CAROTENE-RICH FOODSTUFFS OF VEGETABLE ORIGIN.

The work was undertaken with a view to find out the source or sources of foodstuffs which are cheap and at the same time rich in carotene-precursor of vitamin A. The results would be helpful in fortifying the hydrogenated fats and vegetable oils in accordance with the suggestion put forward by I.R.F.A. Nutrition Advisory Committee in July 1946.

Peels from oranges and citrus fruit have been investigated. It was found that these are exceptionally rich in carotene. In Table II are shown some results of analyses.

TABLE II.

Carotene content of orange and sweet lime peels.

(μg. per 100 gm. of the fresh peel.)

Fruit.	Treatment prior to drying.		
	Blanched for ½ min.	Autoclaved.	Vaccum desiccated.
Orange			4550
	..	..	12155
	11133	5166	13466
	7533	4466	5466
Sweet lime ..	11600	6960	9640
	5160	7480	..
	12600	8320	7880

The solvent partition technique coupled with the chromatographic studies have shown that about 55 to 60 per cent of the carotenoids is β-carotene.

Some experiments were further carried out to evolve means and ways for the storage of these rinds without the loss of vitamin. The results, however, showed that the various methods so far adopted (such as blanching, autoclaving and drying in vaccum) have not been able to check the destruction. Further work including the feeding trials, is in progress.

(6) Enzymic liberation of nicotinic acid.

It is universally accepted that the acid hydrolysis of vegetable foodstuffs for the estimation of nicotinic acid by colorimetric procedure is full of pitfalls. It is known that under such circumstances various chromogenic substances are also extracted which interfere with the development of the specific nicotinic acid colour, on the treatment with cyanogen bromide and aniline hydrochloride. The microbiological assays using Lacto-bacillus casei or arabinosus or enzymic hydrolysis by using hog's stomach mucosa are some other methods which have been lately suggested.

Investigations have therefore been undertaken at this unit to evolve a method for the estimation of nicotinic acid by enzymic hydrolysis using rats' stomach and intestinal mucosa. The enzymic liberation is done at various pH ranges and time intervals comparable with physiological process within the body. The work is in progress.

(7) Research on vitamin D.

Experiments conducted during the last year have thrown doubts on the identity of serum phosphatase and bone phosphatase in ricket in rats. Rats were kept on ricketic diet of different $\frac{Ca}{P}$ ratios. Their serum phosphatase estimations were done at different intervals by taking small amount of blood from the heart. It was found that as the rickets developed the serum phosphatase values instead of showing increase showed gradual decrease. The bone phosphatase, however, did not show any marked change. Further experiments are in progress to understand more about this phenomena in rats.

14. Enquiry into estimation of vitamin A activity of plant foods under Dr. Bashir Ahmad at the laboratories of the Council of Scientific and Industrial Research, University Buildings, Delhi.

The primary aim of this investigation has been to make a more exact determination of the vitamin A activity of plant foods, than has been possible by the methods commonly employed, by isolating and estimating the individual carotenoid pigments present and by taking into account the biological activity of the different carotenoids involved. The technique of chromatographic separation was used, the pigments were identified by means of their absorption spectra, and the estimation made colorimetrically. The more common foodstuffs have been studied, including green leafy vetegables, coloured root vegetables, coloured fruits and a few carotenoid-containing animal foods. Some

of the important and rich sources of carotenoids have been examined more intensively by studying the several varieties occurring commonly.

In the last report, the carotenoids occurring in seven leafy vegetables, three varieties of carrots, eleven varieties of mangoes and eleven varieties of oranges were reported. The work has been continued in all the different varieties of common foodstuffs. The different pigments isolated from the 26 leafy vegetables have now been identified. These are xanthophyll, neo-xanthophyll, β-carotene, neo-β-carotene U, and neo-β-carotene B. Of course, the principal pigments were xanthophyll and β-carotene. The remaining three pigments namely neo-xanthophyll, neo-β-carotene U and neo-β-carotene B are present in relatively small proportions, the total quantity not exceeding 4·3 per cent. The total carotenoid content of the different leafy vegetables show a range of 123·8 μg./g. - 517·6 μg./g. Rape seed, bathu, karam, fenugreek, bengal gram, coriander, mint, celery, beet root, radish, turnip, cauliflower and knol khol leaves are among the richest sources, and lettuce, spinach, purslain or kulfa, betel and cabbage leaves are moderately rich. The etiolated inner leaves of cabbage contain very small amount of carotenoids.

In the case of 19 other vegetables examined, the amount of carotenoid pigments present are much smaller as compared with leafy vegetables. Nevertheless, in these vegetables also, xanthophyll and β-carotene constitute the principal pigments being 0·7 - 30·5 μg./g. and 0·17 - 11·5 μg./g. respectively. Neo-xanthophyll and neo-ρ-carotene U have been found to be absent in most of the vegetables. In the few cases in which these pigments have been observed, neo-xanthophyll varied between 0·06 - 3·10 μg./g.; that of neo-β-carotene U 0·28 - 0·55 μg./g. and neo-β-carotene B 0·1 - 1·3 μg./g. In this class of vegetables, pumpkin, ridge gourd, peas, parmal, broad beans (tender pod), cluster beans, lobia, green chillies and pumpkin contain higher amount of carotenoids, whereas ladies' fingers, bitter gourd, knol khol, tinda, cauliflower, cauliflower stem, french beans, broad beans (matured pod) are among the poor sources.

The amount of total active pigments in most of the leafy vegetables varied between 70 - 120 μg./g. In case of others, the total active pigments varied between 1·96 - 12·3 μg./g. for vegetables containing low amounts of carotenoids, and 0·17 - 3·71 μg./g. for those which are exceedingly poor sources.

The vitamin A potency was found to be 110 - 241 I.U./g. for rich sources of leafy vegetables and 39·7 - 89·4 I.U./g. for the moderately rich sources. In case of non-leafy vegetables, vitamin A potency was found to vary from 3·3 - 20·5 I.U./g. for vegetables containing higher amount of carotenoids, and 0·3 - 5·6 I.U./g. for the poor sources.

Carrots, being one of the rich sources of carotenoids, its investigation has been extended to 14 varieties including three reported earlier. The colour of different varieties ranged from pale yellow, pink, and orange to deep red and violet. Seven different pigments were identified viz. xanthophyll, γ-carotene, neo-β-carotene U, β-carotone, neo-β-carotene B, α-carotene, and neo-α-carotene. In the red varieties, the main pigment is of course β-carotone varying from 40·4 - 90·9 μg./g. constituting 60 - 86% of the total pigments while α-carotene was present to the extent of 10·2 - 10·3 μg./g. representing 9·3 - 23·0%. The quantity of neo-β-carotene B was also appreciable being 5·0 - 10·0 μg./g. or 7·9 - 16·2% of the total. Xanthophyll, γ-carotene, neo-β-carotene U and neo-α-carotene are all present in small proportions up to 3·2 %.

In case of orange coloured carrots, α-carotene was found to be the principal pigment and was present from 13·6-26·0 μg./g. constituting 34·0-51·9% of the total pigments. The amount of β-carotene in these varieties varied from 4·2-16·0 μg./g. or 13·3-32·5%. Unlike red coloured carrots which contained xanthophyll only in small quantities, 2·2-3·2% the orange varieties contained this pigment quite in appreciable quantities, 5·1-9·9. μg./g. or 10·2-29·3%. The remaining pigments, neo-β-carotene U, neo-β-carotene B and neo-α-carotene were present only in small quantities. These can serve as a source for isolating α-carotene.

It was interesting to note that in pale yellow to deep yellow and pink varieties, xanthophyll was found to be the principal pigment occurring between 2·0-13·2 μg./g. or 75·9-93·4% of the total pigments. The next pigment of importance was β-carotene though only small quantities are present, 0·2-2·3 μg./g. or 5·0-18·4%. In this group, γ-carotene, α-carotene and neo-α-carotene were generally not detected except in the case of yellow and deep yellow varieties.

In the case of violet coloured carrots also, xanthophyll was found to be the principal pigment and was present from 2·1-7·0 μg./g. or 84·7-93·7%. Like pale and light yellow varieties, γ-carotene, α-carotene, and neo-α-carotene were not detected. The amount of β-carotene was exceedingly small varying between 0·2-0·7 μg./g. or 6·3-11·9%.

The vitamin A potency has been found to vary 85-195 I.U./g. in case of red varieties of carrots, 22-51 I.U./g. in case of orange, 0·3-4·4 I.U./g. in case of pale yellow to deep yellow and pink varietes and 0·3-1·2 I.U./g. in case of violet varieties.

In the fruits, three categories were studied, mango, loqat, plum, peach, and water melon as example of rich sources of carotenoids; papaya, apricot, oranges among the moderately rich, bananas, grapes as poor sources. The carotenoids of ten different varieties of mangoes, one of the richest sources, were reported recently in the Ind. Jour. Med. Res. **34,** 69, (1946). Ten more varieties have now been examined. The main pigments are xanthophyll and β-carotene constituting 21-65% and 25-60% of the total carotenoids respectively. In smaller quantities three other isomers are present. In peaches, xanthophyll is present in rather large amounts (63-87%) while in water melon, the main pigment is lycopene (90-99%), β-carotene being present only in traces. Papayas were found to contain 31-60% of a carotenoid which is not identical with any common pigment and we have not yet been able to identify it. The traces of pigments in banana and grapes are mainly xanthophyll and β-carotene.

Ten different varieties of tomato representing cross breeds between the commercial and the wild varieties have also been studied. Pigments present are xanthophyll, lycopene, neolycopene, β-carotene and neo-β-carotene B. The amount of the principal pigment, lycopene, in the different breeds varied between 63·6-181·8 μg./g. while β-carotene was only between 5·9-16 μg./g. It was interesting to observe that those which contained larger amounts of β-carotene were poor in lycopene.

A number of egg yolks have also been examined. Five different pigments have been observed, namely lutein, zeaxanthin, cryptoxanthin, β-carotene and pseudo-α-carotene; zeaxanthin forming 20-30% and lutein 56-69% of the total pigments.

On the basis of the amount of individual carotenoids present in various fruits, the vitamin A activity of eightyseven foodstuffs have been calculated. It has been stated that the method which has been followed gives a more accurate estimate of the vitamin A activity of the plant foods. It is now possible to assess the percentage of error introduced in various foodstuffs if the vitamin A activity is calculated on the basis of total hydrocarbon pigments present being regarded as β-carotene. In some foods, it is found that the differences by the two methods are not very marked. For the majority of green leafy vegetables, the latter method gives only 3-7% higher results. For other vegetables constituting the stem, flower and other portions of the plant, the differences are appreciable and vary between 18-30%. In fruits also, the differences are of the same order. In some fruits and vegetables such as carrots, peaches and loqats etc. the common method gives results which are higher by as much as 30-58%.

The development of carotenoid pigments during ripening of fruits was studied in the mango and the tomato. It was found that while all the pigments are synthesized during ripening, β-carotene in the case of mango and lycopene in the case of tomato increase at a much greater rate than xanthophyll and other pigments. The ratio of xanthophyll to β-carotene which is above one in the unripe mango, being generally in the neighbourhood of 1·5 gradually decreases to 0·3 or even less during the process of ripening. The synthesis of β-carotene proceeds at the rapid rate of 5·0-10·0 μg./g. of tissue in 24 hours. Thus an average sized mango may synthesize 1,200 μg. of β-carotene in a day. The rate of synthesis, of course, varies in different varieties within a certain range.

15. Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

1. *The nature of iron in lecitho-vitellin.*

It has been previously reported that total iron in egg yolk can be recovered in the lecitho-vitellin protein fraction. In order to study the nature of iron in the lecitho-vitellin, following investigations have been carried out :—

(i) Whole of the iron in lecitho-vitellin protein fraction is present in ferric state and cannot be dialysed out, but when the iron of lecitho-vitellin is reduced with acetic acid and sodium hydrosulphite, the whole of iron can be dialysed out and made to combine with αα′-dipyridyl to form a water soluble dialysable ferrous complex.

(ii) The liberation of iron from lecitho-vitellin by :—

(a) Alkaline hydrolysis—Treatment of lecitho-vitellin with 0·5 N NaOH on water bath for three hours or on keeping overnight under alkali of the same strength at room temperature, liberates the whole of iron from protein fraction. After completion of hydrolysis, protein substance or substances, precipitated out with trichloro acetic acid, contained no iron. All the iron goes to the hydrolysate.

(b) Acid hydrolysis—Hydrolysis of lecitho-vitellin with hydrochloric acid (1 : 1) for three hours on water bath liberates in the hydrolysate only 10-16 per cent of the total iron in the original sample.

2. *The fate of different added ions to egg yolk and lecitho-vitellin protein.*

Like egg yolk, a colloidal solution of lecitho-vitellin in 10 per cent NaCl can take up added iron in ferric state and the added iron cannot be recovered in the protein free filtrate. Again like egg yolk, it fails to take up divalent iron or copper. Whether this adsorption of ferric iron by egg yolk or lecitho-vitellin is specific for iron or characteristic of other trivalent metal ions was next studied. It was observed that various divalent ions such as manganese (Mn), magnesium ($Mg^{\cdot\cdot}$), calcium ($Ca^{\cdot\cdot}$), zinc ($Zn^{\cdot\cdot}$) were not taken up either by egg yolk or by a colloidal solution of lecitho-vitellin; on the other hand, both egg yolk and lecitho-vitellin could take up added trivalent metal ions such as chromium ($Cr^{\cdots}$) and aluminium ($Al^{\cdots}$). Thus adsorption of metal ions by egg yolk or by lecitho-vitellin is not specific for ferric iron but a general phenomenon shared by other trivalent metals.

3. *Iron of goat liver.*

It has been reported by Granick (J. Biol. Chem. 1942, **146**, 451) that horse-spleen contains an iron bearing protein complex 'ferritin'. Like spleen, liver is a rich source of iron and so fractionation of liver protein was undertaken in order to isolate a fraction rich in iron and to investigate the nature of iron in the fraction. A brief account of the method of fractionation with slight modification from that of Granick is given below :—

An aqueous extract of liver (150 cc. per 100 gm. of minced liver) was heated to 60° C; water soluble protein thereby coagulated and was centrifuged off. A clear red brown solution remained. 30 gms. of solid ammonium sulphate were then added to each 100 cc. of the solution the resulting precipitate was kept in cold storage at 0°C. overnight, next centrifuged down and dissolved in minimum amount of distilled water. In order to precipitate " ferritin " protein fraction, 4-5 gms. of cadmium sulphate ($CdSO_4$. 8/3 H_2O) were added per 100 cc. of the solution. Precipitation of the complex started immediately and the precipitate was centrifuged down. Yield : on wet basis 1·4 gm. of " ferritin " per 100 gm. of fresh liver. On analyses, it has been found to contain iron (54-78 mg. %) and nitrogen (9·4-12·4%).

(a) Nature of iron on " ferritin" complex :—All the iron in ferritin complex is in ferric state; of this about 90-92 per cent is ionisable.

(b) Normally, iron of " ferritin " cannot be dialysed out; but it can be dialysed out on reduction.

(c) Enzymic hydrolysis :—Enzymic hydrolysis with pepsin or papain does not liberate iron in the hydrolysate. ,,

It appears from results obtaind by this enquiry that iron in most food-stuffs is in ferric state and all the ionisable iron is not biologically potent. As in case of egg yolk, all the iron present is ionisable, but it is not biologically active as indicated by haemoglobin regeneration. Many cereals and other food stuffs also contain a good amount of iron. This will have considerable bearing on applied nutrition in the study of what fraction of iron present in such cereals is biologically active. It is, therefore, proposed to continue the scheme on the following lines :—

(i) Biological availability of iron in the lecitho-vitellin fraction of egg-yolk :—It has been observed by Sherman *et al.* (J. Biol. Chem. 1934, **107**, 289) that though egg-yolk contains ionisable iron, it is supposed

to be not fully utilised in haemoglobin regeneration due to the fixative effect of sulphur of the egg-yolk on copper which helps mobilisation of iron required for haemoglobin regeneration. Lecitho-vitellin contains no sulphur (Plimmer, J. Chem. Soc. 1908, **93**, 1500). So it is of interest to investigate whether the iron of egg-yolk in lecitho-vitellin is more potent in haemoglobin regeneration than iron of whole egg-yolk which is supposed to contain an inhibitory factor, such as sulphur. The fate of total iron (a) portion biologically potent (b) portion biologically stored in egg-yolk and lecitho-vitellin when taken as food, is being studied by (a) haemoglobin regeneration in the nutritional anaemia of rats and by (b) feeding and balance sheet experiment.

(ii) The complex "ferritin" isolated from goat liver has been shown to be rich in iron. The biological availability of this iron both from the standpoint of haemoglobin regeneration and of body storage is also being followed up.

16. Enquiry on poor rice diets and food preparations in common use in South India under Dr. Eleanor D. Mason at the Women's Christian College, Madras.

(1) Rat growth experiments.

(a) *Comparison of rice before rationing and rationed rice.*

Analysis of growth records for 9 weeks of young albino rats of comparable initial weights fed on the basal poor rice diet (90 per cent parboiled rice cooked) during the years 1944 to 1946 gave the following results :—

Table I.

Comparison of effects of rice diet during pre-rationing and rationing periods in Madras.

	Pre-rationing period.	Rationing period.
Growth—per cent increase over initial weight	35·5 (107 rats)	56·4 (56 rats)
Food consumption gms. per rat per day	29·8 (22 rats)	41·0 (30 rats)
Per cent increase per gms. food consumed	1·18	1·37

Thus the less milled rice of the controlled, rationing period stimulated appetite, accelerated growth, and produced better growth per gm. food consumed than the highly milled rice of the period before rationing. On the rationed rice the initial loss of weight usually observed when weanling rats are transferred from stock diet to the rice diet did not appear.

This enforced change in the basal diet affected subsequent studies of the effects of supplements.

(b) *Studies of supplements to rice diets and their inter-relations.*

Fat :—The previously reported (I.R.F.A. Reports, 1944, 1945) deleterious effects of even small quantities of fat (·5 gms. per day) with the poor rice

diet and the further decrease in growth with increasing fat have been consistently confirmed, with butter, " vegetable ghee ", Dalda, and groundnut oil. The only exception has been two out of many experiments with butter, in which there was a moderate acceleration of growth with the butter supplement. This was probably due to a greater amount of vitamin A in the butter as well as of the vitamin of the B-complex in less-milled rice. Experiments of Mason, Purushottam and Tew in 1945-46 at the Harvard School of Public Health in Boston, U.S.A., with rice diets showed a growth-promoting effect of *American* butter, but a significantly negative effect of groundnut oil (unpublished data, paper in preparation). In these experiments both vitamin A and riboflavin were found to exert highly significant positive effects on growth, with rice diets, especially when in combination.

Calcium :—The highly significant growth-promoting effect of calcium lactate (·115 gms. per day) previously reported has been consistently confirmed.

Fat-calcium inter-relation :—Four experiments involving 48 rats with butter (·5 gms. daily) and calcium lactate (·115 gms. daily) separately and combined confirmed the findings of Mason, Theophilus and Frimodt-Moller (I.J.M.R. 1945, **32**, 219) that there is no inter-action between butter and calcium. The effects of these supplements on relative growth rates (related to initial weight) over a period of 9 weeks as compared with the unsupplemented rice diet were : butter −2·45, calcium lactate +5·41, both highly significant, the inter-action −0·48, of no significance, the standard error being ±0·615.

Protein :—Mason, Purushottam and Tew (cited above) found that vitamin-free casein at levels of 5 per cent and 10 per cent in the rice diet stimulated growth markedly at first, but that growth then ceased abruptly and the animals died sooner than the animals on the basal rice diet alone. There was strong evidence of acute deficiency of vitamin A in these rats. Experiments in Madras with certain proteins—purified casein, Parry's food yeast, de-fatted mutton—have shown a high rate of mortality after 5 to 9 weeks strongly suggestive of a similar harmful effect of at least some proteins as single supplements to rice diets. These effects have so far appeared only with small animals (initial weight around 35 gms.) but not with rats of initial weight around 50 gms.

Fat-protein inter-relation :—Single experiments combining Dalda and yeast, " vegetable ghee " and yeast, ground-nut oil and yeast, " vegetable ghee " and purified casein, and butter and defatted mutton, have not shown any inter-relation of any of these proteins with fat. In the experiments of Mason, Purushottam and Tew (cited above) growth was more inhibited by the combination of vitamin-free casein and groundnut oil than with either supplement alone or with no supplements. These rats developed symptoms of deficiencies of both vitamin A and riboflavin that appeared to be accentuated by the presence of casein and oil.

Adexolin and riboflavin :—In a single small-scale experiment with 8 rats adexolin at the level of 1 drop a week providing approximately 50 I.U. of vitamin A and 8 I.U. of vitamin D per day stimulated growth silghtly. Riboflavin (from Upjohn's riboflavin tablets dissolved in water given so as to supply 40 micrograms per day) did not stimulate growth at all beyond the basal diet and both rats with riboflavin alone died within 7 weeks. They showed skeletal deformities in spine and thorax causing pressure upon heart and lungs and markedly reducing the thoracic cavity. The *combination* of adexolin and ribo-

flavin gave excellent growth and normal skeletal development. This very small-scale experiment is being repeated and when supplies of vitamin A apart from D and pure riboflavin are available these will be substituted.

Adexolin and casein :—A small-scale experiment with a high level of adexolin (1 drop per day) and purified casein (·4 gms. per day) showed a highly significant interaction between adexolin and casein. This also needs confirmation.

These studies with separate and combined supplements to the highly deficient poor rice diet tend to show that the addition of *single* food factors may actually be harmful, as in the case of fat and certain proteins, perhaps they increase the requirements for other factors already deficient. There is some evidence that vitamin A and riboflavin *in combination* are particularly important supplements. Further experiments are in progress and planned, to clarify the problem of the inter-relationships of fat, protein, vitamin A, riboflavin and calcium.

(2) Analysis of South Indian food preparations.

The following data complete and supplement previously reported analyses (I.R.F.A. Reports, 1944 and 1945).

Table II.

Analysis of South Indian food preparations.

Sample.	Moisture. g. %	Protein. g. %	Fat. g. %	Mineral Matter. g. %	Calcium. g. %	Phosphorus. g. %	Iron. mg/100g	Calories. per 100 g
Bajjis—vegetable plantain—								
Home made sample	40·58	5·603	24·16	2·315	·051	·152	11·58	236
Bajjis—onion—								
Home made sample	47·77	5·64	18·49	2·63	·059	·114	16·21	202
Bajjis—brinjal—								
1 Hotel sample	43·31	6·30	18·40	2·75	·058	·130	10·22	202
Pagoda—(Bengal gram, raw rice, onions, green chillies ginger, curry leaves, coriander leaves, salt, water, gingelly oil)								
1 Hotel made sample	15·25	8·18	40·70	2·73	·015	·039	11·32	276
Home made sample	8·70	10·62	21·58	2·83	·042	·149	17·66	293

(3) Analysis of common South Indian green leafy vegetables.

The following greens commonly eaten in South India have been analysed. All greens with the exception of Paruppu keerai and radish tops were collected from among the weeds in the college compound, the two samples being collected from different parts of the compound. Two analyses were made of every sample. Only edible portions were used—thorns in Nerringi and flowers and pods of Vellakeerai and flowers of Kuppameni being discarded.

TABLE III.

Analysis of common South Indian green leafy vegetables.

Name of Green.	Sample.		Moisture gms. %.	Protein gms. %.	Ether extractions gms. %.	Total Ash gms. %.	Calcium gms. %.	Phosphorus gms. %.	Iron mgm/100 gms.	Calories/100 gms.	Vit. C mgm/100 gms.
Paruppu keerai (Portulaca oleracea).	3 Samples		94·11	1·671	—	2·408	·084	·051	25·09	35	—
Radish Tops (Raphanus sativus)	2 ,,		90·37	2·43	—	2·295	·289	·039	17·83	46	—
Kuppameni (Acalypha indica).	Sample	A	85·25	6·58	1·55	3·315	·679	*·058	16·97	63	147·9
	,,	B	75·83	6·91	1·27	2·965	·57	·141	17·65	66	—
		Mean	80·54	6·74	1·41	3·14	·62	·099	17·31	64	147·9
Mukarratai (Boerhaavia repens)	,,	A	82·56	6·74	·71	4·60	·651	·102	18·31	*59	27·23
	,,	B	86·50	*5·44	1·09	4·06	·684	·097	18·47	·72	—
		Mean	84·53	6·09	·90	4·31	·667	·099	18·39	65	27·23
Pacharisi keerai (Euphorbia hirta).	,,	A	77·66	5·01	1·72	2·76	·464	·067	20·44	116	44·32
	,,	B	78·62	4·30	1·71	3·54	·629	·146	22·04	*125	—
		Mean	78·14	4·65	1·71	3·15	·546	·106	21·24	120	44·32
Nerringi (Tribulus terristris)	,,	A	79·19	7·61	·62	4·54	1·40	·074	9·66	92	41·53
	,,	B	78·99	*8·01	·46	4·21	1·71	·090	8·79	*96	—
		Mean	79·09	7·81	·54	4·37	1·55	·082	9·22	94	41·53
Vellakeerai (Cleome viscosa).	,,	A	76·22	6·21	2·07	4·20	·982	·072	22·95	104	—
	,,	B	84·60	5·08	1·66	3·31	·780	·074	25·95	83	—
		Mean	80·41	5·64	1·85	3·75	·881	·073	24·45	93	—
Suravallai keerai (Trianthema monogyna).	,,	A	91·56	2·42	·45	2·05	·071	·037	39·39	39	—
	,,	B	91·10	1·75	·33	3·19	·042	·018	36·69	42	—
		Mean	91·33	2·09	·39	2·62	·056	·027	38·54	41	—
Punnaku keerai	,,	A	80·32	5·82	·67	*2·46	·251	·037	32·58	—	—
	,,	B	80·89	6·43	—	2·56	·250	·039	38·72	—	—
		Mean	80·60	6·12	·67	2·51	·250	·038	35·65	—	—
Kuppa keerai (Amaranthus viridis).	,,	A	81·30	5·06	—	2·48	·318	·050	16·39	—	—
	,,	B	82·20	5·29	—	*3·20	·322	·055	20·98	—	—
		Mean	81·75	5·17	—	2·84	·320	·052	18·74	—	—

* Only one analysis was made.

17. Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary protein to blood and muscle haemoglobin under Professor M. Damodaran at the University Biochemical Laboratory, Triplicane, Madras.

Experiments were described in the last annual report on comparative efficiencies in blood regeneration of casein, egg albumin, wheat gluten and rice protein fed at a uniform level of 5% to animals suffering from experimental anaemia, and further, on the effect of feeding two of these proteins, casein and egg albumin at varying levels. During the year under report experiments on these lines have been continued with other proteins and at higher levels of intake. Work has also been carried out for evolving a suitable technique for determining the whole of the haemoglobin including that of blood and muscle, in experimental animals.

The results of experiments in which the proteins of meat and liver were tested for their haemopoietic activity are given in Table I. The experimental procedure was the same as that previously described, the animals being made anaemic by injection of phenylhydrazine and the recovery from anaemia studied on diets incorporating 5% of the protein under test. Beef was used as the source of meat protein. Minced beef was coagulated by boiling with water and defattened first with alcohol and finally with petroleum ether. As a source of liver protein minced ox-liver was used. The minced liver was suspended in water, and after adjusting the pH to 4·5 with dilute hydrochloric acid, was coagulated by heating to boiling point. The material obtained was defatted by alcohol and petroleum ether. The protein content was based on the total nitrogen of the dried powder.

TABLE I.

Increase of R.B.C. and Haemoglobin in a period of 8 *days at* 5% *levels of dietary protein.*

	Meat Protein.				Liver Protein.			
	4th day	12th day	Inc.	% Inc.	4th day	12th day	Inc.	%Inc.
R.B.C. in Millions per c.mm. ..	3·36	5·99	2·63	78·3	3·59	5·54	1·95	54·3
Haemoglobin in gms. per 100 c.c. ..	8·43	14·47	6·04	71·6	8·54	13·22	4·69	54·7

The figures given are for the first part of the experimental period for eight days from the onset of severe anaemia (fourth day after injection of phenylhydrazine). Between these two proteins of animal origin the protein of whole muscle meat proved to be decidedly superior to the proteins of liver. Thus on a diet containing muscle protein, in the first 8 day period from the stage of acute anaemia the increases in haemoglobin and R.B.C. were 71·6% and 78·3% respectively; the corresponding values for liver protein were 54·7% and 54·3% respectively.

In the studies on the influence of level of protein on blood regeneration already casein and egg-albumin upto a level of 20% were tried. The experiments were continued with diets containing 25% and 30% of casein.

TABLE II.

Percentage increase in R.B.C. and Haemoglobin in 8 days at different levels of casein.

Expt. No.	Protein level.	No. of animals.	% increase in R.B.C.	% increase in haemoglobin.	No. of days taken to come to normal.
1.	5%	6	62·1±5·1	59.5±2.8	12
2.	15%	6	86·1±3·8	74.7±1.82	10
3.	20%	6	100 ±3·76	75.6±1.6	8
4.	25%	6	100·8±4·21	77.9±1.8	8
5.	30%	6	105 ±5·7 (4 Days)	86.7±2.4 (4 Days)	4

The results (Table II) are interesting inasmuch as they show abrupt increases in haemoglobin formation at certain levels. Thus on raising the protein intake from 5 to 15% the increase in haemoglobin is raised from about 60% to 75%. With increases from 15 to 25% the rate of haemoglobin regeneration remains fairly constant. But at an intake of 30% there is again an accelerated haemoglobin formation the increase being about 90% and the initial values being reached in four days after the stage of acute anaemia as against 8 days at lower levels. This would indicate that various aminoacids required for haemoglobin building reach their optimum levels successively with progressive increase in protein intake. These results are important and require to be repeated for confirmation.

Experiments to study the effect of different levels of wheat gluten and meat protein were continued. These proteins were tried at 5, 15 and 20% levels, and the results are summarised in Table III.

TABLE III.

Increase in R.B.C. and Haemoglobin in 8 days at different levels of wheat gluten and meat protein.

Expt. No.	Protein level.	No. of animals.	R.B.C. in millions per c.mm. 4th day	12th day	Inc.	% inc.	Haemoglobin in gms. per 100 cc. 4th day	12th day	Inc.	% inc.	No. of days taken to come to normal
1.	Protein Free	6	3·31	4·09	0·78	23·6	8·19	10·81	2·62	32·0	25
Wheat gluten.											
2.	5%	6	3·48	4·75	1·27	36·5	8·98	12·41	3·43	38·2	20
3.	15%	6	3·58	5·34	1·76	49·2	8·98	13·67	4·69	46·9	11
4.	20%	6	3·53	5·97	2·44	69·1	8·52	14·19	5·67	67·5	11
Meat Protein.											
5.	5%	6	3·36	5·99	2·63	78·3	8·43	14·47	6·04	71·6	12
6.	15%	6	3·71	6·90	3·19	86·0	8·50	15·17	6·67	78·5	8
7.	20%	6	3·80	7·36	3·56	93·7	8·40	15·38	6·98	83·1	8

Determination of total haemoglobin (blood+muscle) in normal animals.

There are no methods available in the literature for the determination of total haemoglobin content of experimental animals. A modification of the method described by Abderhalden for the determination of blood volume of small animals (Zeit. f. Biol. 1902, **39**, 197) was employed to give what appears to be a satisfactory procedure for the extraction of both blood and muscle haemoglobin.

The animal was killed and skinned, and the alimentary tract dissected out. The carcass was then thoroughly minced in a meat mincer. After experimenting with various media such as water, saline, phosphate buffer etc. dilute pyridine was found to be the most suitable material for extracting the haemoglobin from the mince. The extraction with aqueous pyridine was continued till the final extract failed to give a test for pyridine haemochromogen on treating with sodium hydroxide and sodium hydrosulphite. The extract was filtered through drill and finally through two layers of filter paper, made up to volume and the haemoglobin determined in an aliquot by the pyridine haemochromogen method of Rimington (British Medical Journal, 1942, Vol. I, p. 177). The total haemoglobin content of normal rats as determined by this method averaged 747 mg. per 100 g. body weight. The average blood haemoglobin for rats is given by Somogyi (Nature, 1936, Vol. 138, p. 763) as 630 mg. per 100 g. body weight so that the muscle haemoglobin amounts to about 117 mg. per 100 g. body weight. Before this method can be applied for studying the influence of protein on total haemoglobin production it will have to be checked by spectrographic determination of blood and muscle haemoglobin separately.

18. Enquiry on the preparation of anti-rachitic substances under Professor B. V. Bhide at the Maharajah Pratap Sinh Chemical Laboratory, S. P. College, Poona.

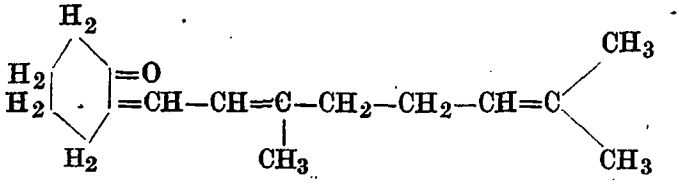
The object of this enquiry is to prepare substances similar in structure to calciferol. The work of this enquiry was started in June, 1946.

There are three characteristic points in the structure of calciferol viz. (i) the presence of a triene system, (ii) a cyclopentano cyclohexane ring and (iii) a long side chain attached to the cyclopentano ring. A research scheme was planned so as to prepare substances having these characteristics.

With a view to synthesise substances having a triene system, attempts were made to condense cyclohexanone with cinnamic aldehyde. But even under a variety of conditions cyclohexanone and cinnamic aldehyde in the presence of aqueous sodium hydroxide gave invariably dicinnamilidene cyclohexanone m.p. 184. This substance has been analysed and characterised through its semicarbazone. However, in the presence of petroleum ether it has been found possible to get cinnamilidene cyclohexanone m. p. 113 in a very small yield.

Cyclohexanone has been condensed with citral and citralydine cyclohexanone has been isolated in the pure condition. It has been characterised by

its ioxime and semicarbazone. This substance by Reformatsky's reaction with ethyl bromoacetate, followed by hydrolysis gave the following compound.



Another series of experiments were carried out to synthesise rings C and D in calciferol. With this end in view attempts were made to prepare 4 methoxy hydrindone by cyclising 2-methoxy-phenyl-propionic acid. Several attempts with the usual cyclising agents failed to give the desired product. A synthesis of the 4-methoxy-hydrindone is being attempted by the cyclisation of the dibasic acid obtained by the oxidation of 5-methoxy-tetralone.

Attempts were also made to prepare cyclopentano cyclohexane by the Stobbe condensation of cyclohexane 1 : 2-dione with diethyl succinate. It was found, however, that the yields of the dione obtained by the oxidation of cyclohexanone by selenium dioxide were very poor. Moreover one of the assistants fell ill due to selenium poisoning. The experiments, therefore, had to be given up.

With a view to prepare a hydroaromatic substance corresponding with hexoestrol, 4-methoxy-2-methyl-cyclohexanone carboxylate has been prepared. This is expected to give the desired product in three steps. Work on these lines is in progress.

19. Enquiry on the nutritive value of soya-bean milk under Dr. S. S. De at the Indian Institute of Science, Bangalore.

Composition :—The composition of soya milk varies according to the variety used for the preparation of milk. The total solids ranged from 8·0 to 10·55 per cent. Usually varieties having less fat content produce milk of low solid content. Addition of sodium bicarbonate or disodium hydrogen-phosphate during boiling increases the solid content of the milk. Incubation of the paste mixed with water containing 0·1 per cent sodium chloride at 60-70° C. for 2-3 hours increases the protein content of the milk. The taste of the milk also varies with the variety.

Effect of germination on the biological value of the proteins of soya milk :—The biological value of the proteins of soya milk prepared after overnight steeping is 1·8 gms. and that of cow's milk is about 2·0 gms. as determined by growth method on rats fed at 10 per cent protein level. The biological value of protein of the milk prepared from 2 days' germinated beans was found to be 2·1 gm. This showed that soya milk protein is not deficient in regard to any essential amino acid. When the milk was given at 5 per cent protein level, the biological value was found to be 1·15 gm. but raw soya-bean fed at that level failed to maintain the growth of the rats.

Fortification with calcium :—The calcium content of soya milk is about 20-30 mgms. per 100 cc. ; the phosphorus content is about 60 mg. per 100 cc.

Fortification of the milk has been tried with calcium carbonate, lactate, gluconate and glycerophosphate. With these salts the calcium content of the milk could be raised to 50-55 mg. Addition of a little dextrin helps in keeping the milk stable. The best result was obtained with calcium hydrogen phosphate. The calcium content could be raised to 90 mg. per 100 cc. The calcium and phosphorus metabolism of soya milk fortified with this salt has been studied and it was found that both calcium and phosphorus are absorbed to the extent of 85 per cent. The addition of calcium hydrogen phosphate slightly affects the taste of the milk and on repeated boiling, it has a tendency to settle down.

Total nutritive value of soya milk :—Freshly weaned rats were fed on soya milk as their sole diet. Another group was fed on cow's milk. Daily small doses of copper, manganese and iron salt were given to each rat. The experiment was conducted for a period of three months. The growth rate in the case of soya milk was about 70 per cent of that obtained with cow's milk. The soya milk used was not fortified with calcium.

In vitro digestibility of soya milk :—Although the *in vivo* digestibility of soya milk is of the same order as that of cow's milk, the *in vitro* digestibility of soya milk as determined by peptic followed by tryptic digestion is very low. The tryptic digestion rate of soya milk is about 25 per cent of that of the cow's milk. It has been found that the low tryptic action is due to the presence of trypsin inhibitor which is present in raw soya-bean. The amount of inhibitor present in the milk is about 1/6th of that present in raw bean.

Supplementary effect to poor rice diet :—The supplementary effect of soya milk prepared from overnight steeped bean, germinated beans, soya milk fortified with calcium, soya milk fortified with yeast and calcium has been compared with that of cow's milk. The following results are illustrative :—

		Gm. gain in weight per rat per week.
(a)	Poor rice diet	3·5
(b)	,, +10 cc. soya milk (soaked overnight) ..	6·6
(c)	,, +10 cc. soya milk (germinated 48 hours) .	7·2
(d)	,, +above fortified with calcium ..	8·1
(e)	,, +germinated soya milk fortified with calcium and yeast	9·5
(f)	,, +10 cc. cow's milk	8·6

From the above results it appears that soya milk fortified with calcium and yeast extract can even surpass the supplementary effect of cow's milk.

Balancing with other oilseeds :—Guha in Calcutta and Ahmad at Lahore prepared a mixture from soya-bean, groundnut and malted barley for use during Bengal famine. A palatable milk from the above materials has also been prepared here with some alteration in the method of preparation. The proportions used were soya 60 parts, groundnut 25 parts and malted barley 15 parts. The milk has a malt flavour and the soya flavour is nearly masked.

Vitamin content :—Vitamin content of soya milk varies with the variety used for preparing the milk, carotene being most variable. The range is from 200 I.U. to 800 I.U. of carotene per litre of milk. The vitamin C content of soya milk has also been determined in several varieties and it has been found that it varies from 22 mg. to 40 mg. per litre of milk. The most important

feature of vitamin C in soya milk is that it is not destroyed by exposure to sunlight whereas, vitamin C in cow's milk is completely destroyed under the same conditions. Soya-beans contain appreciable amount of vitamin E and a large part of it is extracted in the milk. Investigation is in progress to see to what extent it helps in the utilisation of content in the milk.

Comparative trials with soya-bean milk and cow's milk in child welfare centres.

The total number of children under experiment was over 120. Of these, only 97 continued to take the two milks for the whole period, so only the observations relating to the weights of these children have been included. The increase in the weights of children are recorded in the following table.

Average weight increase in different age groups.

Infants (two months to eleven months).	Number of subjects.	Average increase in weight.
Cow's milk	11	2·61 lbs
Soya-bean milk ..	10	2·71 lbs.
Toddlers (under 3 years).	**Number of subjects.**	**Average increase in weight.**
Cow's milk	22	2·51 lbs.
Soya-bean milk ..	19	1·54 lbs.
Toddlers (under five years).	**Number of subjects.**	**Average increase in weight.**
Cow's milk	17	1·85 lbs.
Soya-bean milk	18	2·15 lbs.

Feeding of soya curd and rice to school children :—The Bangalore C. & M. Station is providing curd and rice as free mid-day meal for 5,000 school children. Each child is being given 8 oz. cooked rice and 4 oz. curd. Four hundred pounds of curd per day are provided for feeding 1,600 children in 17 primary-schools, the rest of the children are being given the equivalent amount of skimmed milk curd. Only 60 children in each group are under observation. The experiment has been in progress for the past three and a half months.

Large-scale production of soya milk :—The large-scale production of soya milk has been standardised. The equipment in the department supplemented by those that have been added is adequate to produce over 1,000 lbs. of milk per day. The present daily production is about 550 lbs. The semi-large scale trials have yielded a good deal of valuable data bearing not only on the equipment needed but also the cost of production of soya milk.

LEPROSY.

20. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

The work during the year has included clinical, therapeutic, histological, bacteriological and immunological studies.

THERAPEUTIC.

(1) *Promin and Diasone :*—A start has been made with the study of the two sulphone drugs promin and diasone, in the treatment of leprosy. The investigation has been started on suitable cases of the lepromatous type All are in-patients in the Albert Victor Hospital, Calcutta. Since the drugs are toxic (the most marked toxic effect being the destruction of blood), the treatment is regulated by periodic blood examinations. When indicated by the findings of the blood examinations the patients are given iron and/or liver in addition to the sulphone drugs.

The results so far are encouraging ; during the six months that the patients have been under treatment most of the patients have shown clinical improvement, although none of them have become bacteriologically negative. In a few patients the treatment had to be discontinued because they could not stand it. Diasone has an advantage that it can be given by mouth while promin has to be administered intravenously since it is very toxic when given orally.

(2) *Stilbamidine :*—Last year it was reported that stilbamidine was effective in preventing the generalised infection in rats inoculated with rat leprosy material, if the treatment is started immediately after inoculation. During the present year, the curative value of the drug has been investigated in rats in which the infection was allowed to get established before treatment was started. The results indicate that stilbamidine delays the progress of the generalised infection, but does not have a curative effect. This work will be discontinued.

(3) *Hydnocarpic derivatives of sulphonamides :*—The possibility has been investigated of incorporating the active principles of hydnocarpus oil in the sulphonamide molecule, which may result in the production of a substance more effective in the treatment of leprosy than either of the two remedies alone. Dr. Rajagopalan prepared the following compounds :—N^4-Butyryl-N^1 hydnocarpyl sulphonamide, N^4 caproyl-N^1 hydnocarpyl sulphonamide, N^4—N^1 Dihydnocarpylamido diphenyl sulphone, $4 : 4^1$-dihydnocarpylamido diphenyl sulphone, 4 Nitro-4^1 hydnocarpyl-amido diphenyl sulphone. These compounds have been tested *in vitro* for their inhibitory action on cultures of acid-fast bacilli with discouraging results. This work will be discontinued.

(4) *Streptomycin :*—An anti-biotic substance obtained from cultures of the fungus *Actinomyces griseus*. Streptomycin is known to inhibit the growth *in vitro* of the tubercle bacillus, and other acid-fast bacilli. It has been reported to inhibit the experimental tuberculosis in guinea-pigs, and to act favourably in patients suffering from tuberculosis of the genito-urinary tract, and suppurative lymphandenitis. The drug has obvious potentialities in the treatment of leprosy.

Attempts at procuring specimens of streptomycin for trials in leprosy having failed, cultures were obtained of *Actinomyces griseus* with the object

of producing small amounts of streptomycin for trial in experimental rat leprosy. A preliminary study has been completed of the optimum conditions for the growth and anti-biotic production. It has been possible to overcome the initial difficulties of making the fungus grow well and produce anti-biotic fluid of high potency. The results of this study may be summarised as under:

(i) The addition of hay infusion to the medium used by Waksman greatly improves the growth and the anti-biotic activity. Replacement of glucose in the medium with sucrose is also beneficial.

(ii) Incubation in the cool room gives decidedly better results than incubation at the ordinary temperature. A cold incubator at about 24°C. would be best for the purpose.

(iii) The highest activity is found in culture-fluids 10 to 12 days old.

(iv) Filtration through an L3 candle reduces considerably the activity of the fluids obtained from cultures of *A. griseus*.

(v) It is now possible to obtain a titre of 1 in 460 against *Bact. coli* the activity against acid-fast bacilli is even higher. Concentrates from the filtrates have been obtained with a titre of 1/10,000. This material will be used for trials in rats experimentally infected with rat leprosy.

(5) *Aspergillin*:—An anti-biotic obtained from cultures of *Aspergillus fumigatus*. It is known to inhibit the growth of *Myco. phlei* and the human and bovine strains of the tubercle bacillus. A study was started to investigate the therapeutic value of this substance in rat leprosy. Ten strains of *A. fumigatus* have been obtained from the National Collection of Type Cultures, London. One of these strains, strain No. 367, has been found to show the greatest activity, but even with this strain it has not been possible to raise the titre of the fluid beyond 1 in 55. It is believed that the strains under study are not very active, and it is therefore proposed to discontinue the study till more active strains are available.

(6) *Asiaticoside*:—A glucoside isolated by French workers in Madagascar from the plant *Hydrocotyle asiatica*. These workers reported very favourably on the treatment of leprosy with this glucoside. The plant *Hydrocotyle asiatica* grows abundantly in India, and attempts have been made to isolate the active glucoside in collaboration with the Professor of Chemistry, School of Tropical Medicine, Calcutta. A small amount of the substance has been obtained which is insoluble in water, slightly soluble in alcohol, and freely soluble in pyridine. However, the substance has been found to be inferior to the ordinary sulphonamides in inhibiting the growth *in vitro* of cultures of acid-fast bacilli. Prof. B. V. Bhide of the S. P. College, Poona, has isolated a glucoside from this plant. The *in vitro* tests on this substance have also given discouraging results. This work will be discontinued.

Clinical.

(1) *Cases of leprosy of 'doubtful' classification*:—A large majority of cases of leprosy can be classified as 'neural' or 'lepromatous' on clinical grounds. There are, however, a small percentage of cases in which the clinical findings are not clear cut; it is difficult or impossible to make a proper classification or prognosis in these cases. The lesions in these cases resemble the tuberculoid lesions in being localised, but are smooth and shiny with ill-defined margins and smears from them show moderate to large number of leprosy bacilli.

With the object of studying the course of the disease and assessing the prognosis, a number of such cases have been kept under a long-term study. Several of these cases have now been under study for over three or four years, and repeated bacteriological, immunological and histological examinations have been made on each case. The study has so far shown that in some of the cases the atypical clinical and histological findings are temporary, and are often associated with the phase of reaction; in some other cases, however, the atypical findings persist.

(2) *Erythrocyte sedimentation rate*:—A study has been completed of the value of erythrocyte sedimentation rate as a help in regulating the dose of the hydnocarpus preparations in the treatment of leprosy. The test is of no value for this purpose.

(3) *Eye lesions in leprosy*:—A study has been started of the eye lesions in the different types of leprosy in collaboration with Capt. E. J. Somerset, Prof. of Ophthalmology, Medical College, Calcutta. Eyes are liable to be affected in both the main types of the disease, but the nature of the lesions in the two types is different. There is considerable evidence that eye complications in leprosy are much less serious in India than in some other countries. The study had to be discontinued when Capt. Somerset went on leave to England. The study has now been resumed on his return.

BACTERIOLOGICAL.

(1) *Cultivation of the leprosy bacillus*:—Attempts have been made to grow the leprosy bacillus on egg media enriched with the enzymes oxidase and peroxidase. No macroscopic growth or microscopic evidence of multiplication of the seeded bacilli has been obtained. It has, therefore, not been possible to confirm the report of Triana (a worker in Cuba) that the leprosy bacilli grow easily on the ordinary egg media used for the tubercle bacillus, provided some enzymes are added to it. It is now proposed to repeat the work of Dr. R. Row, who has reported successful cultivation of the leprosy bacillus in symbiosis with leishmania culture.

(2) *The value of nasal smears*:—A study has been completed of the frequency with which leprosy bacilli are found in the nasal mucosa of the 'neural' cases of leprosy with bacteriologically negative skin lesions. Althouth nobody now subscribes to the view that the initial lesion of leprosy is in most cases found in the nose of the patients, there appears to persist an impression in the minds of some leprosy workers that the leprosy bacilli may often be found in the nose of persons with bacteriologically negative skin lesions. The present investigation has shown this belief to be wrong. During the year about 2,000 "new" cases of leprosy of the neural type attended the out-patient clinic of the leprosy department. In 1,750 of the cases multiple smears from the skin lesions were negative for leprosy bacilli, while in the remaining 250 cases few to a fair number of leprosy bacilli were found in the skin lesions. Leprosy bacilli were found in only 2 of the 1,750 cases with bacteriologically negative skin lesions, and in 80 of the 250 cases with bacteriologically positive skin lesions. It is thus rare to find leprosy bacilli in the nose in the absence of the bacilli in the skin. The above 2 were cases of reaction in which there was an acute exacerbation of the disease.

It can be concluded that although the finding of leprosy bacilli in the nose in the absence of bacilli in the skin is not a common feature, it is important to

make a bacteriological examination of the nose in addition to the skin lesions, before declaring a case "closed" or non-infectious.

Immunology.

(1) *Antigens from acid-fast bacilli :*—A study has been completed of the acid-soluble protein fractions of cultures of some acid-fast bacilli. The study was made in the hope that an antigen suitable for work in leprosy may be isolated from culture of an acid-fast bacillus. This hope has not materialised, although in the beginning the results were encouraging.

(2) *Specificity of the lepromin test :*—Fernandez, a South American worker, had reported that filtrate from lepromin produced uniformly negative results in non-contacts, implying thereby that the filtrate provided a specific test for leprosy. This matter was investigated in some villages in the Punjab where the disease does not exist in an endemic form. The results do not confirm the report of Fernandez.

PLAGUE.

21. Plague researches under the Director, Haffkine Institute, Bombay.

(I) Plague vaccine.

Keeping qualities of the casein hydrolysate plague vaccine:—The comparative tests to estimate the keeping qualities of agar-grown and casein hydrolysate plague vaccines were continued. Tests after 20 months' storage at 37° C have been completed with the following results:—

	Mouse Protective Dose.			
	Casein hydrolysate vaccine.		Agar vaccine.	
	at 0° C.	at 37° C.	at 0° C.	at 37° C.
Fresh vaccine	0·003 c.c.	..	0·004 c.c.	..
After 2 months' storage ..	0·003 c.c.	0·003 c.c.	0·004 c.c.	0·007 c.c.
„ 4 „ „ ..	0·004 c.c.	0·004 c.c.	0·004 c.c.	0·006 c.c.
„ 8 „ „ ..	0·004 c.c.	0·004 c.c.	0·004 c.c.	0·006 c.c.
„ 14 „ „ ..	0·0046 c.c.	0·0044 c.c.	0·0038 c.c.	0·0055 c.c.
„ 20 „ „ ..	0·0046 c.c.	0·014 c.c.	0·0056 c.c.	0·015 c.c.

The above results have a very important bearing on the expiry time of the vaccine.

(II) The effect of DDT dust on the breeding of the Indian rat-fleas, *Xenopsylla cheopis* and *X. astia*. (Report on the work done by Dr. M. Sharif, Officer-in-Charge, Bombay Government Plague Recrudescence Inquiry in Sholapur and adjoining districts).

Both the jar and burrow experiments with 10 per cent DDT in pyrophylite reported in the last year's report were continued. In the former 12,799 fleas and in the latter 4,200 fleas were utilized. In order to ascertain the lethal effect of the DDT dust on rat-fleas, attention was paid to their behaviour under the influence of DDT, and its residual effect and minimum lethal dose for them; for these factors enable to determine the concentrations and quantities of DDT required for their control under diversity of situations in which they are found.

DDT is a slow killing contact insecticide. When adult fleas come in contact with the DDT dust, the well-defined symptoms that occurred in succession in them are (1) excitement, (2) loss of jumping power, (3) loss of crawling power, and (4) stoppage of kicking of legs. Every lethal concentration of DDT killed all the fleas within a day; but the rapidity with which earlier two symptoms occurred, depended upon its concentration. Thus the behaviour of fleas towards DDT will help in assessing roughly the concentration of its different formulations in the field.

In the jar experiments, a concentration of 1 in 10,000 continued to kill 100 per cent rat-fleas up to 396 days tried after dusting the DDT powder. In the control experiments the mortality rate varied from 3 to 33 per cent. A very negligible deterioration in the immobilizing effect of DDT, as indicated by the delay in inhibition of the jumping power of all the fleas, with the length of

exposure to natural elements occurred; but this was only confined to the uppermost layer of mixture of the DDT dust and sand.

The minimum lethal dose for the adult fleas of *X. cheopis* was 0·005 mg. of DDT per gram of sand, as 99 per cent of the rat-fleas died 238 days after mixing it with the DDT dust. Thus even a concentration of 1 in 200,000 gave a fairly long residual effect for adult fleas; but higher concentrations only helped in the early immobilization of their jumping power. A concentration of 1 in 300,000 was, however, not totally effective after 125 days.

In the artificial, W-shaped, about twenty-one feet long burrows, when dusted with 37-79 g. of 10 per cent DDT powder with a calcid baby duster or cyanogas "A" foot pump duster, the results were not so consistent as in the jar experiments. In the individual experiments, the percentages of mortality on the total and the recovered fleas respectively were 58-100 and 97·8-100 within 428 days tried in the first arm up to a distance of about 5 feet, 68-96 and 81·3-100 within 425 days in the second arm up to a distance of about 10½ feet, 50-100 and 54·4-100 within 397-420 days in the third arm up to a distance of about 15½ feet, and 30-96 and 33·3-100 with 230-336 days in the fourth arm up to a distance of about 20½ feet. The discrepancies met in the burrow experiments lend to reasonable analyses which incidentally throw light on the different difficulties likely to be encountered, whilst dusting natural rat burrows for flea control, and on the steps to be taken to overcome them in field operations.

Less than 100 per cent success in the mortality of fleas, obtained in many cases in the artificial burrows, is more due to the disturbing factors than to the deterioration of DDT on account of volatilization, as is clearly borne out by 100 per cent mortality of fleas in jar experiments for almost as long a period as in the burrow experiments. The disturbing factors were unequal distribution of the DDT dust by the ordinary dusters used, resulting in a very poor dispersal of the dust in the last two arms of the W-shaped burrows, and not mixing of the dust with the finely powdered soil in them. Equal distribution of an adequate amount of the DDT dust in the rat burrow and its proper mixing with the burrow debris are very necessary. In order to do so the use of a power duster, such as is made by the application of a dusting mechanism to the exhaust pipe of a jeep, will be very helpful and economical.

In order to ascertain the effect of DDT on different stages of rat-fleas, 1 or 5 g. of the 10 per cent DDT dust were thoroughly mixed with 615 to 965 g. of debris of about one year old breeding jars, containing many thousands of adults, cocoons, larvae and eggs of *X. astia*. All the adults and larvae of the existing generation were killed within a day. Only recently emerged adults and hatched larvae were seen alive, which ultimately died. Thus DDT is not ovicidal, and it has no effect on the pupæ well protected within cocoons; but adults and larvae are highly susceptible to it. On account of the residual effect of DDT, the recently hatched larvae and emerged adults were killed off after coming in contact with the DDT dust.

22. Plague enquiry at Coonoor in the Nilgiris district under the Director of Public Health, Madras.

The Hosur plague enquiry was closed by the end of May 1946 and the new plague enquiry in Nilgiris district was started by the beginning of August.

Accommodation for laboratory and office of this new enquiry was provided by the Director, Nutrition Research laboratories, Coonoor. The work, with which this scheme has been started is intensive cyanogas fumigation of selected villages in the district and this has been going on for the last 5 months.

During these few months of preliminary arrangements, it has not been possible to commence work on all the different lines of work proposed. This is due to the fact that no facility is available in the present premises for conducting any experiments on live rats or fleas for want of a properly protected animal room and also because that the small laboratory staff including a II class Health Officer has not been appointed yet to assist in the laboratory work of the Special Plague Officer who is in sole charge of the plague control measures in the Nilgiris district.

Plague in the Nilgiris :—Plague prevails in the Nilgiris district throughout the year. In the plains plague is generally more a rural problem, but in the Nilgiris district, the urban areas of Ootacamund and Coonoor which together comprise about 1/50th the total area and ¼ of the population of the district have had about 50% of the total deaths.

During the period under report Coonoor town was passing through a mild epidemic of plague and 26 attacks and 22 deaths have been reported. Ootacamund municipality was free from indigenous plague during the period. From 20 villages and hamlets in the rural areas of the district 81 attacks and 37 deaths were reported. The epidemic was comparatively mild during the 3rd quarter. Besides these villages which reported human plague there were 59 villages and hamlets which reported rat fall and in 41 of these the infection was proved positive to plague by examination of dead rodents. In one of the villages 5 cases of pneumonic plague broke out during the course of the bubonic plague epidemic and all these cases died.

Progress of activities.

Cyanogas fumigation :—Cyanogas fumigation was done in all the infected villages and also in the threatened villages around them. Wholesale fumigation was resorted to in all the premises where heavy rat infestation was suspected or where recent rat falls had occurred. Market stalls, godowns, grain-shops, etc., in Ootacamund and Coonoor municipalities were fumigated wholesale once every month. Fumigation of congested residential areas in these towns are also being done once every 2-3 months. Only 2 human cases occurred in 2 out of the 41 villages which were fumigated soon after detection of rat plague. The epidemic was mild in all villages where fumigation was started after the occurrence of human cases.

Experiments with DDT as a pulicide :—Experiments were carried out in order to study the effect of DDT as a pulicide.

1. In one eries of experiments 332 rat holes were sprayed with 5% DDT solution in kerosene oil by means of a stirrup pump and blocked with earth. The sprayed rat holes were opened at varying intervals to study the residential effect of DDT.

(a) From 39 rat holes excavated after 2 weeks 10 rat nests were obtained, of which 3 rat nests yielded 26, 16 and 2 live fleas respectively.

(b) From 20 rat holes excavated after 3 weeks 5 rat nests were obtained of which 2 yielded 5 and 9 live fleas respectively.

(c) From 43 rat holes excavated after 4 weeks 9 rat nests were obtained and in 3 of these, there were 3 and 5 and 7 live fleas present.

(d) 35 rat holes were excavated after six weeks and live fleas 3-10-7 were obtained from 3 rat nests out of 8 rat nests obtained from the burrows.

(e) 12 rat nests were obtained by excavation of 45 rat holes after 8 weeks. 3 of these yielded 2-8-11 live fleas.

It was found that out of the 182 rat holes excavated at varying intervals from 2 to 8 weeks, there were live fleas in 14 rat nests out of 44 nests obtained.

During the process of excavation of these burrows it was noticed that the burrows were tortuous and that the solution of DDT was confined to the first few inches of the burrow and did not reach the actual site of the rat nest to be effective. Those rat nests which were directly sprayed on with the solution of DDT did not yield any fleas.

It may, however, be stated that the action of DDT cannot be compared with that of cyanogas fumigation as the former at best kills only the rat fleas and that only when they are directly sprayed on, unlike cyanogas which kills both the rats and fleas by the H.C.N. which can penetrate any sort of tortuous rat holes.

2. In the 2nd series of experiments 10 houses which were heavily infested with pulex irritants (human fleas) were sprayed with 5% DDT solution on the floor and up to a height of 2 feet on the wall. The effect was very good both from the experience of the occupants as well as from observations made in these houses.

It is proposed to extend the field trial of DDT on a wider area and determine the rat flea index at different intervals to see how far the rat flea index will be affected.

CLINICAL RESEARCH.

23. Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

Vitamin B levels in oral cancer and vitamin B deficient people.

Vitamin levels of nicotinic acid, riboflavin and thiamine before and after the administration of these vitamins (simultaneously) were determined in (a) sixteen oral cancer patients, some of whom presented clinical signs of avitaminosis B, (b) sixteen persons who sought advice for degenerative or chronic inflammatory lesions in the mouth suggestive of mild vitamin B deficiency or a precancerous condition without any clinical or histological evidence of cancer and (c) sixteen healthy laboratory workers. The lowest excretion levels for nicotinic acid, nicotinamide methochloride, thiamine and riboflavin were determined from the study of the sixteen healthy persons and are shown in Table I.

TABLE I.

The suggested control levels of the vitamins before and during test dose of the vitamins.

	Nicotinic acid.	Nicotinamide methochloride.	Thiamine.	Riboflavin.
Before test dose ..	15·4	6·4	0·295	0·705
After test dose ..	34·7	14·6	1·320	1·740

The lowest excretion of the vitamins for the normal group has been taken as the suggested control levels.

The average excretion levels before, during and after administration of test doses of these four substances in the three groups are shown in Table II. These values show a significantly lower range in the intermediate group and oral cancer patients.

TABLE II.

Average excretion levels of the vitamin, 16 *persons in each group* (1) *before* (2) *during and* (3) *after the administration of vitamins* (100 *mgms. Nicotinic acid,* 10 *mgms. Thiamine and* 12 *mgms. Riboflavin.*

	Nicotinic acid.			Nicotinamide* Methochloride			Thiamine.			Riboflavin.		
	1	2	3	1	2	3	1	2	3	1	2	3
Normal	20·00	43·50	23·40	7·60	19·00	10·40	0·272	1·570	0·810	0·855	2·400	1·240
Intermediate	11·80	23·30	13·30	5·40	8·70	5·82	0·135	0·730	0·300	0·620	1·190	0·735
Cancer	6·55	12·76	7·80	2·63	5·94	3·16	0·103	0·467	0·226	0·367	0·903	0·480

*F_2 factor detected by Najjar and others, now established as nicotinamide methochloride.

Studies on experimental production of cancer of the liver by feeding p. dimethylaminoazobenzene (Butter Yellow).

Experimental production of cancer of liver by butter yellow (p. dimethylaminoazobenzene) feeding of rats of two different strains has been started.

Twelve groups of eight animals each with additional controls are being fed a standard diet with a known amount of the carcinogen. Two groups are sacrificed at intervals of one month for a biochemical and histological study of the liver during the course of tumour development. These studies include a determination of (1) the respiratory enzymic activity (2) the role of sulfhydryl compounds in the metabolism of the carcinogen (3) the relation between the sterols, phospholipids and fatty acids.

24. Enquiry on Naga sore under Dr. S. R. Pandit at the Pasteur Institute, Shillong.

During the period under report eleven patients with active Naga sore were admitted to the clinical research hospital attached to the Pasteur Institute and kept under observation for varying periods. Six of these were from Tea Estates in the Lakhimpore district; 3 from Khasi Hills district and two from Kamrup. In all the patients the ulcers were distributed over the lower extremities. Seven patients had a single ulcer whereas the remaining four had multiple ulcers ranging from two to four. Seven gave a history of cut and injury which were the starting points of the ulcer and in two the ulcer appeared spontaneously as a bleb after initial itching. In the remaining two, however, the sore arose from lesions due to scabies. There was evidence of malaria in two cases. In the peripheral blood of two other cases *W. bancrofti* was detected without any clinical manifestation of the infection. With regard to helminthic infestations six were negative, one had ankylostomes and four others had other helminths. One of the cases gave a strong positive while three others gave doubtful Wassermann reactions.

There were no definite signs of malnutrition in any but more than half of these cases suffered from anaemia in a moderate degree.

Microscopic studies :—Smears stained by different stains and dark ground examinations were systematically carried out in each of the patients from day to day. In the acute stage of the ulcer fusiformis bacilli were found constantly in enormous numbers and usually predominated. The number of this organism decreases with the age of the ulcer until, in the healing ulcer it was difficult to find any. Spirochaetes were detected in four cases. It is noteworthy that in cases where spirochaetes were absent from the start at no stage of the disease could their presence be detected. Gram positive Cocci were seen in all the cases; besides in a few, diphtheroids and proteus vulgaris were met with. A gram negative slender curved bacillus reported before was seen in three of the cases.

Cultural study :—The evidence collected so far suggests bacillus fusiformis to be of aetiological significance. Difficulty is still being experienced in the isolation of the organism. In certain cases primary growth of the organism was obtained but it failed to grow in subcultures. With a view to find out a suitable improved medium, growth-requirements are being studied. Two strains of fusiform bacillus isolated from cases were studied in detail. The organism is a strict-anaerobe and grows at 37° C. It is gram negative in cultures non-motile and pleomorphic. The fusiform shape is less evident after subcultures but the beaded appearance could be well demonstrated with the use of Giemsa stain. The colonies are round with an entire margin, clear and tiny,

resembling streptococcal colonies. For primary isolation 5% CO_2 was used but satisfactory growth was obtained in an atmosphere of hydrogen only after a few subcultures.

Biochemical reaction :—The organism did not ferment glucose or any of the sugars used i.e. lactose, mannite, saccharose, maltose and saliein. For growth in sugar media addition of blood or serum was necessary. Anaerobiosis was obtained either with the use of Mackintosh & Filde's jar or with the addition of reduced iron to the media. There was indole formation ; gelatin was not liquified.

Agglutination tests were put up with six samples of sera from recovered cases of Naga sore with the suspension of fusiformis. The results were not satisfactory in view of the fact that the suspension was not inagglutinable in normal saline.

Attempts were made to cultivate the spirochaetes but so far no success, has been obtained. The work is being continued.

Experiments on transmission :—A fresh suspension of the discharge from an active sore was injected intradermally into a guinea-pig and a rabbit but it failed to initiate any lesion. The direct application of pus on the scarified skin and scarified sclera of these animals also proved unsuccessful. On the other hand, intradermal inoculation of the pus as well as the direct application on the scarified skin in human volunteers, produced typical ulcers with a preponderance of fusiform bacilli. The pus used, besides bacillus fusiform also showed the presence of gram+cocci and proteus in one instance.

A young culture of the fusiformis was injected intradermally as well as subcutaneously into a recovered case of Naga sore, but no lesion was produced. A killed suspension was injected intradermally in a case with active sore but no allergic reaction resulted. The same suspension instilled into the eye of the same case also failed to produce any reaction. The filtrate from Naga sore discharge and a thick suspension of fusiformis—separately and in combination, were introduced intradermally into three healthy volunteers. The filtrate alone did not produce any reaction, but the culture with or without the filtrate produced a small vescile in 24-48 hours after which they retrogressed without resulting in a sore.

Epidemiological study :—Ten estates in the Dibrugarh area in upper Assam were surveyed. These Estates were not visited before. Seventyfive individuals gave a history of having had the sore in 1945. In the great majority of them, ulcer had healed but few had chronic ulcers. In the series thirtyfour were males and fortyone females. With the exception of six who were factory hands all were engaged in outdoor work i.e. hoeing, pruning, picking etc. There were none in the age group under 10 and there were six in the age groups above fifty. The largest number fell in the age group 10-30 years. The ulcers were single in fifty cases and multiple in the remaining twentyfive. The ulcers were distributed over the lower extremities and in only one person there were two more ulcers as well on the forearm suggestive of autoinoculation. In thirty-nine individuals there was definite history of trauma at the site of ulcers—cuts in 27, leech bite in 10 and bee and wasp bite in 2. The remaining individuals stated that vesicles or papules appeared spontaneously with itching. It was obvious that any breach in the continuity of the skin, however trivial it might be, may initiate the development of the ulcer. There was no evidence to show that an insect vector played any role in the causation of the disease.

There was a crop of cases in the early rains and cases continued during the rainy season but many of the estates which recorded a heavy rainfall escaped the infection entirely. Further two contiguous gardens under similar conditions had different histories, one having several cases and the other none. In one estate there were sporadic cases in 1944 but the disease assumed an epidemic form in 1945.

Newcomers in the area (new labour recruits) were not more susceptible than older residents. The findings of the survey support the previous observation that diet plays little part in the causation of the disease. Although the diet consumed was deficient in essential proteins and vitamins and this has always been so. The incidence of the disease varied during a period covering several years from nil to an occasional outbreak of epidemic proportions. There is little doubt that the spread of the disease in 1942 and 1943 was, to a large extent, due to the infection in labour camps engaged in military projects and the subsequent return of the labourers to their homes in the estates.

25. Enquiry into hypoproteinaemia and other conditions associated with malnutrition under Dr. John Lowe at the School of Tropical Medicine, Calcutta.

(a) *Studies of simple methods of blood protein estimation* :—The copper sulphate specific gravity method of estimating total blood proteins was adopted as a routine. By this method findings in apparently healthy individuals were in grammes per cent :—

	Maximum.	Minimum.	Mean.
Total plasma protein ..	8·0	6·8	7·5
Albumin	5·4	4·5	4·8
Globulin	2·2	3·3	2·7
Albumin-globulin ratio ..	2·4 to 1	1·4 to 1	1·8 to 1

This work on normals has given a mean protein value of about 7·5 gms., instead of the generally accepted figure of 7·0. The albumin-globulin ratios in healthy persons have been within normal limits indicating that the Biuret reaction is a satisfactory method of estimating fractions for clinical work.

These methods, therefore, show that the results obtained are accurate enough for clinical work although the figures for blood proteins are a little higher than those given by the Kjeldahl and other similar methods. The work has shown, however, that in many tropical conditions and conditions of malnutrition the albumin-globulin is the important point to be studied.

(b) *Application of the methods* :—By these methods about 800 observations have been made in healthy people, in persons suffering from different tropical diseases, in some sick destitutes and persons suffering from malnutrition. The following are the main points brought out in this study :—

(i) In the study of the *normal healthy individuals* no appreciable difference has been observed between protein values of vegetarians and non-vegetarians.

(ii) Blood proteins in certain specified diseases were as follows :—

In *malnutrition* a marked fall in total blood proteins occurs, the degree depending on the severity of the condition.

In *kala-azar* a rise in the total blood proteins occurs due to a marked increase in the globulin fraction. The lowering of albumin is also very marked with the result that the albumin-globulin ratios as low as 0·23 : 1 was recorded with total blood protein within normal limits. Normal or raised total blood proteins with albumin-globulin ratios of less than 1 : 1 is in many cases a strong indication of the presence of kala-azar, and this finding may be of diagnostic value since it appears very early in the disease.

Similar findings but less marked may be found in *chronic tuberculosis*. In chronic *diarrhoeas* and *dysenteries* in the absence of defective diet the blood protein values showed no marked abnormality, contrasting markedly with cases of *nutritional diarrhoea* in which low blood protein values and particularly low albumin values were constantly found. Blood protein estimations are often useful in differentiating between chronic infective dysenteries and diarrhoeas due to malnutrition.

In *cholera* owing to extreme dehydration, blood protein values have risen as high as 15 gms. (twice the normal value). These probably have considerable influence in the causation of anuria seen in cholera and give a strong indication that the treatment of cholera should not include the intravenous administration of plasma proteins.

In *leprosy* of the severe lepromatous type the findings are rather similar to those in kala-azar, high total proteins, high globulin and low albumin contrasting markedly with leprosy of the neural type in which the blood protein values are often within normal limits.

In *anaemias* due to iron deficiency the blood protein values are not markedly deficient, whereas in nutritional macrocytic anaemia the malnutrition is often accompanied by marked changes in the blood proteins particularly a fall in the albumin.

In *diseases affecting the liver* including cirrhosis of the liver which often accompany tropical splenomegaly and in amoebic liver abscess marked rise in globulin and fall in the albumin are often seen. In more acute conditions of the liver such as infective hepatitis these findings are much less constantly made.

In diseases such as *typhus* and *typhoid* the blood proteins findings are not markedly reduced provided that the patient received adequate diet during his illness. An interesting and previously unrecorded finding has been the marked rise in globulin seen in the condition of '*Tropical eosinophilia*'.

One point needs emphasis strongly—the *frequence with which severe hypoproteinaemia has been induced by the restriction of diet under medical advice.* This is particularly seen in diseases of the gastro-intestinal tract, *viz.* peptic ulcer, dysenteries, colitis, etc. Cases of appalling hypoproteinaemia induced by restricted diet with no adequate reason whatever have been encountered, and liberal feeding with no special medical treatment has restored the patients to health.

(c) *Treatment of hypoproteinaemia :*—During the period under review acute inanition due to starvation has not been seen. All cases of hypoproteinaemia have been able to take diet by mouth. This has been so even in cases with severe nutritional diarrhoea. Therefore, oral treatment has been used with, on the whole, satisfactory results. The failure to respond to proper treatment with high protein diet by mouth usually indicated the presence of

some underlying disease condition such as abdominal tuberculosis. The work has tended to support the view that the oral administration of proteins is the best method of treatment. Some experiments with intravenous administration of proteins were conducted. The following being used (i) reconstituted blood plasma, (ii) human albumin, and (iii) protein hydrolysates prepared at the All-India Institute of Hygiene and Public Health. The limited work in this sphere would not justify any definite conclusions. Blood plasma and albumin are of value in the treatment of nutritional oedema by increasing osmosis but their nutritional value would appear to be limited. Mercurial diuretics have also been of great value in combating severe oedema due to hypoproteinaemia.

26. Enquiry into erythroblastosis foetalis and its association with the Rh factor under Lieut. Colonel S. D. S. Greval, I.M.S., Imperial Serologist to the Government of India at the School of Tropical Medicine, Calcutta.

I. Determination of percentage of Rh+/— state of pregnant women attending the antenatal clinic of the Eden Hospital, Calcutta.

II. Intragroup reactions and other anomalous reactions of the blood of pregnant women from the Eden Hospital.

III. Determination of percentage of Rh+/— state of unselected cases from samples of blood received in the Imperial Serologist's Laboratory from different hospitals for other tests.

IV. Study of anti-Rh animal sera perpared in rabbits.

V. Comparison of the anti-Rh animal sera prepared in this Laboratory with anti-Rh human serum obtained from Dr. A. S. Wiener.

VI. Comparison of tube and slide method in haemagglutination for Rh study.

VII. Study of suspected families.

VIII. Determination of the Rh state of obstetric cases sent from Blood Bank before blood transfusion.

IX. Determination of percentage of Rh subtypes of Indians by Dr. Wiener's human anti-Rh sera.

I. Pregnant women (period of gestation 5 to 9 months) attending antenatal clinic of Eden Hospital were selected. Obstetric history as regards previous pregnancy including abortion, miscarriage, malformation and anaemia or jaundice of previous issues and history of previous blood transfusions, if any, suggesting, Rh sensitization were recorded. Bloods were taken both for rbc and sera. Cells were tested for Rh+/— state with animal anti-Rh serum prepared in this laboratory. Both tubes and slides were used. Women of various communities have been tested with the following results:—

	Tube method Indians.		Slide method Indians.	
	Nos.	Per cent.	Nos.	Per cent.
Positive = ..	305	85·7	309	86.9
Doubtful = ..	20	5·7	15	4·2
Negative = ...	31	8·5	32	8.8
Total ..	356		356	

II. Sera from bloods of pregnant women taken 2 hours previously were inactivated and tested for intragroup reaction to detect any anti-Rh or anti-Hr bodies, if present. Each serum was tested against compatible cells of various groups taking 3 samples of cells from different sources of each group, i.e.

Serum a from subject of group B tested against 3 different samples of rbc of group O & B.

Serum b from subject of group A tested against 3 different samples of rbc group O & A.

Serum ab from subject of group O tested against 3 different samples of rbc of group O only.

Serum o from subject of group AB tested against 3 different samples of rbc of group O, A & B.

Method :—3 drops (1 drop=1 /50 cc.) of the serum and cell suspension were mixed in a tube. Agglutination was only looked for by observing crenation and non-fluidity of the edge of the deposit.

Presence of crenation, however, slight and non-fluidity of the edge would determine the positive reaction, i.e. the presence of antibody in the serum. Results tabulated as follows :—

Total cases done=356

Positive reactions in 2 cases :—(i) In a subject of group AB, Rh—, with group O, A, B and AB cells, all Rh+. This serum did not give positive reaction with Rh— cells. This is a case with weak anti-Rh body in the serum. (ii) In a subject of group B, Rh—, with group O & B cells, all Rh+. This is also a case with weak anti-Rh body in the serum.

III. Unselected samples of Indian blood received for other tests were used for the purpose. In this lot citrated samples as a rule were not available in which cases rbc were obtained from clots by washing. Results tabulated as follows :—

	Tube method.		Slide method.	
	Nos.	Per cent.	Nos.	Per cent.
Positive = ..	185	86·45	185	86·45
Doubtful = ..	8	3·75	10	4·68
Negative = ..	21	9·80	19	8·87
Total ..	214		214	

Cases of citrated blood were also available from a jute mill at Kamarhatti. These results are tabulated separately.

	Tube method.		Slide method.	
	Nos.	Per cent.	Nos.	Per cent.
Positive = ..	40	86·12	41	87·16
Doubtful = ..	3	5·32	2	4·28
Negative = ..	4	8·56	4	8·56
Total ..	47		47	

IV. Only rabbits were found to be satisfactory. Antiserum was divided into many lots and could be used for several months, if found satisfactory by trial on 100 cases. Later, other sera were found to be satisfactory or otherwise in comparison with this serum. The dilution of the serum suitable for the test was determined by trial and error. 10 times the maximal non-agglutinating dose for the monkey rbc was found to be more suitable, than the minimal agglutinating dose for obtaining well-defined reaction with the human rbc. The difference between the two doses is very small. The non-agglutinating dose is always next to the agglutinating dose in the usual series of dilution, and can be termed as sub MAD (Minimal Agglutinating Dose).

The highest titre obtained for the monkey cell was 1 in 2,500 and therefore for human subjects 1 in 250. Each sample of blood was tested with 2 dilutions of this anti-Rh serum, i.e. 1 in 125 and 1 in 250. A negative reaction with 1 in 250 was considered sufficient.

Animal anti-Rh serum of rabbit No. 21, titre 1 in 200, was compared with Wiener's Anti-Rho human serum with the following results:—

Tube.			Slide.		
			Positive.	Doubtful.	Negative.
		91	91	Nil	Nil
Positive	=	—	—	—	—
		92	92	Nil	Nil
		1	Nil	1	Nil
Doubtful	=	—	—	—	—
		Nil	Nil	Nil	Nil
		8	Nil	Nil	8
Negative	=	—	—	—	—
		8	Nil	Nil	8
		100			
Total		100			

Slide.			Tube.		
			Positive.	Doubtful.	Negative.
		91	91	Nil	Nil
Positive	=	—	—	—	—
		92	92	Nil	Nil
		1"	Nil	1	Nil
Doubtful	=	—	—	—	—
		Nil	Nil	Nil	Nil
		8	Nil	Nil	8
Negative	=	—	—	—	—
		8	Nil	Nil	8
		100			
Total		100			

This shows that the anti-Rh animal serum prepared in our laboratory gave corresponding results as with anti-Rho human serum from Dr. Wiener's laboratory. Out of 100 bloods tested there was only 1 discrepancy: one blood positive with Wiener's anti-Rho human serum was doubtful with the animal serum.

VI. *Tube method*:—For Rh determination 5 drops each of 2% rbc suspension to be tested and the anti-Rh rabbit immune serum delivered from a calibrated finger teat pipette (50 drops to a cc) are mixed in a 3" × ½" tube. The tube is left at blood heat for 2 hours.

Slide method:—A slide is divided into 3 compartments. First two compartments contain equal volumes of rbc and two strengths of testing serum. Mixing is thorough. The slide is examined after 1 hour (in a moist chamber).

Tube vs. Slide method:—Only one sample of rbc was positive by slide method and negative by tube method out of a total of 565 bloods tested. Of the rest, rbc negative by one method were doubtful by the other and vice versa and also some doubtful by one method were positive by the other and vice versa. These results are tabulated as follows:—

Indian cases.

Slide			Tube		
			Positive	Doubtful	Negative
Positive	=	486	485	Nil	1
Doubtful	=	28	5	23	Nil
Negative	=	51	Nil	2	49
Total		565			

Tube			Slide		
			Positive	Doubtful	Negative
Positive	=	490	485	5	Nil
Doubtful	=	25	Nil	23	2
Negative	=	50	1	Nil	49
Total	=	565			

VII. Twenty-three families contacted through different agencies were studied including children. Families with history of repeated miscarriages, stillbirths or child developing severe jaundice after birth, were selected. Five pairs were found incompatible as to Rh (husband Rh+ and wife Rh−). Of these two cases of erythroblastosis foetalis developing weak anti-Rh agglutinin in mother's blood are reported as below:—

(i) The mother's serum was tested and weak anti-Rh body detected agglutinating all Rh+ rbc of the same group but not touching any Rh− rbc of the same group. The titre fell within a very short time after delivery (1 month). The obstetric history was as follows: 1st child normal, living. 2nd, 3rd, 4th and 5th children died of other causes. 6th and 7th children died of severe jaundice within eight days after birth.

(ii) The mother was found to be Rh— and her serum contained very weak anti-Rh agglutinin. The obstetric history was as follows:—1st child premature (8 months), died after birth. 2nd child—jaundice noticed on the second day after birth and the child died on the third day.

VIII. Blood from 8 obstetric cases (recipient) was sent from Blood Bank to determine their Rh state before transfusion.

Case 1:—1st gravida carrying 8 months. Anaemic. Blood was Rh+. Transfusion with compatible Rh+ blood was given without any reaction. Living baby was delivered normally in due time.

Case 2:—1st gravida aborted in the 3rd month. Blood was Rh±. Transfusion with compatible Rh— blood was advised. Transfusion was postponed as suitable Rh— compatible blood was not available then.

Case 3:—1st gravida aborted in the 4th month. Shed had some reactions (e.g. chill, rigor and slight rise of temperature but no haemoglobinurea) when transfused with compatible blood whose Rh state was not tested, a fortnight ago. Blood was Rh—. No antibody detected in the serum. Transfusion with compatible Rh— blood was given without any reaction.

Case 4:— 3rd gravida carrying 5 months. Anaemic. Blood Rh—. No antibody detected in the serum. Transfusion could not be given as suitable compatible Rh— blood was not available.

Case 5:—13th gravida. Anaemic. Previous pregnancies normal. Carrying full term. Blood Rh±. Transfusion with compatible Rh— blood was advised. Transfusion was not necessary as she was normally delivered of a living child.

Case 6:—3rd gravida carrying 6 months. Anaemic. 1st child living. 2nd pregnancy terminated in abortion in the 5th month. Blood Rh+. Transfusion was given with Rh+ compatible blood without any reaction.

COMMENTS.

1. In all pregnant cases blood should be tested for determining the Rh state before any blood transfusion, as a routine.

2. All Rh± recipients should be considered as Rh— when any transfusion is to be given to them.

3. All Rh± donors should be considered Rh+.

4. There should be a list of Rh— blood donors of different groups in all maternity hospitals and Blood Banks who should be available at a few hours notice.

IX. 100 unselected samples of blood were tested each with anti-Rh′, anti-Rho and anti-Rh″ sera and the results tabulated as follows:—

Subtypes	Reactions with			Percentage
	Anti-Rh′	Anti-Rho	Anti-Rh″	
rh ..	−	−	−	= 9
Rho (rho)	−	+	−	= 3
Rh′ ..	+	−	−	= Nil
Rh″ ..	−	−	+	= 2
Rh_1 (Rho′)	+	+	−	= 84
Rh_2 (Rho″) ..	−	+	+	= 2
Rh′ Rh″	+	−	+	= Nil
Rh_1 Rh_2	+	+	+	= Nil
			Total	100

27. Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

(A) The preparation of an antigen from the culture of *Malassezia* (Pityrosporon) *ovalis* and the study of its value for complement fixation test in cases of Seborrhoeic dermatitis occupied the first three months of the year as a continuation of the previous year's work.

Two antigens (7th and 8th of the full series) were prepared from the dried powder of the fungus *M. ovalis*. These were ripened for three months and the attenuation or otherwise of their anticomplementary activities were studied every week together with the variation of their antigenic properties with the following results :—

ANTIGEN No. 7.

1st to 4th week—anticomplementary upto 1/8th dilution.

5th to 11th week—anticomplementary upto 1/5th dilution.

12th to 16th week—anticomplementary upto 1/4th to 1/5th dilution.

The result of complement fixation test with antigen No. 7 :—During the first six weeks with 1/10th dilution of the antigen—

Number of cases

(Seborrhoeic dermatitis) — 21 ; Positive — 9 ; Negative — 12.

During the second six weeks with 1/6th dilution of the antigen —

Number of cases

(Seborrhoeic dermatitis) — 15 ; Positive — 8 ; Negative — 7 .

Number of control

(Non-seborrhoeic dermatitis) — 10 ; Positive — 2 ; Negative — 8.

ANTIGEN No. 8.

1st to 4th week — anticomplementary upto 1/3rd dilution.

5th to 12th week — anticomplementary upto 1/4th dilution.

Complement fixation test was done in 29 cases of seborrhoeic dermatitis with the following results :—

Number of cases—29 ; Positive—13 ; Negative—16.

Control cases— 10 ; Positive— 3 ; Negative—7.

Further experiments were discontinued as the results were not encouraging.

With the technique employed the complement fixation test for seborrhoeic dermatitis has not been found to possess any diagnostic value.

(B) Study of the fungus diseases of the nails and the surrounding tissues.

During the last 9 months 209 cases of diseases of the nails and surrounding tissues (perionychia) were studied. This included :—

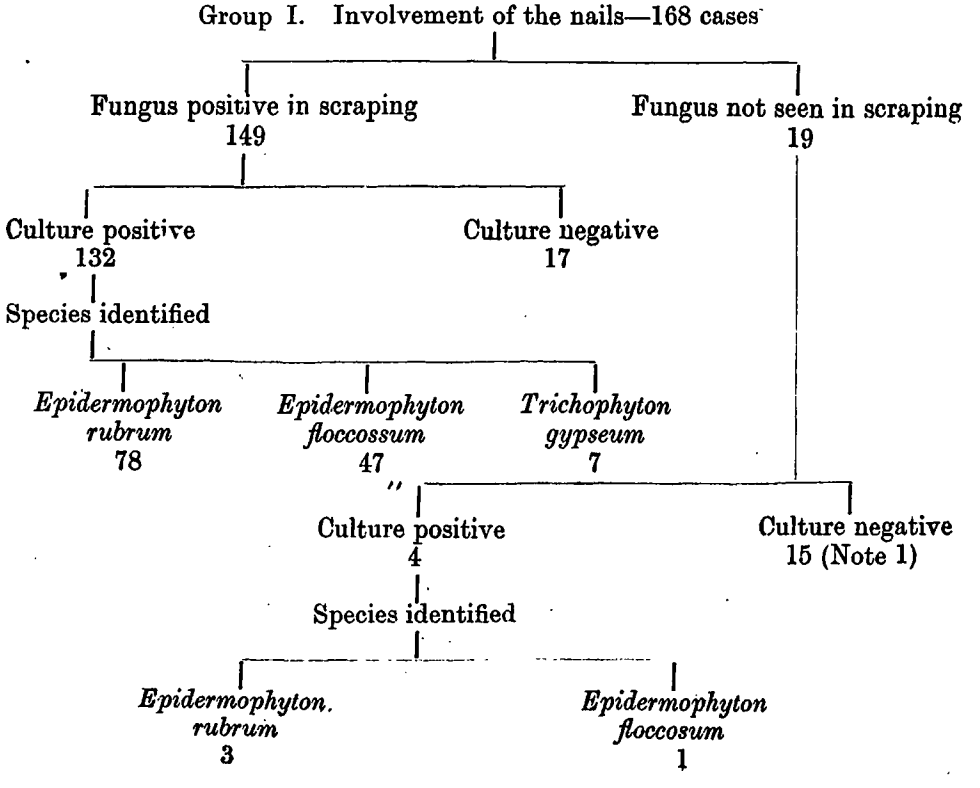
(1) Detection of the fungus infection under microscope.

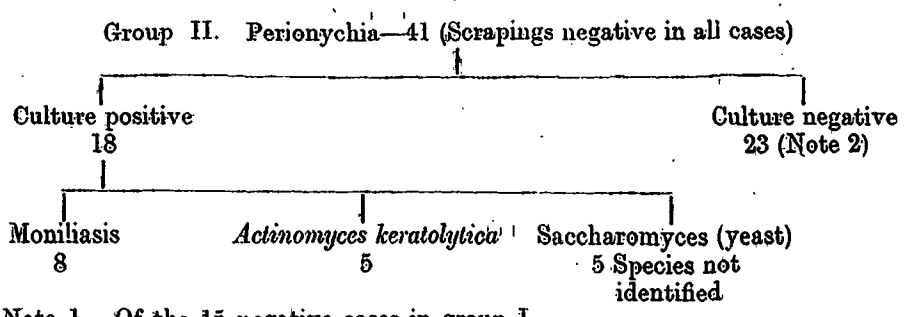
(2) Culture and isolation of the causative organisms.

(3) Identification of the species.

The results are tabulated :—

Number of cases studied involving the nails and the surrounding area	209
Number of cases in which the nails only are involved ..	168
Number of cases of perionychia	41





Note 1— Of the 15 negative cases in group I
11 cases were of psoriasis of the nails, and
4 cases remained undiagnosed.

Note 2— Of the 23 negative cases in group II
20 cases were allergic dermatitis,
2 cases were syphilitic perionychia and
1 case was tubercular perionychia

The study is still being continued.

From the studies made so far it seems that in the affection of the nails the fungus responsible, in order of prevalence, are *E. rubrum*, *E. floccossum* and *Tr. gypseum* and the chief causative organisms for perionychia are Monilia, *Actinomyces keratolytica* and Yeasts.

28. Enquiry into the infections of the feet under Rao Bahadur Dr. M. G. Kini at the Stanley Hospital, Madras.

A STUDY OF THE ANATOMY OF THE FOOT WITH REFERENCE TO "INFECTIONS OF THE FOOT".

Cross-section anatomy.

The work was started on the 1st July 1946. Cross sections were taken of a preserved foot and leg at various levels (16 sections) starting from the webs of the toes to the middle of the leg. The sections were studied with a view to define various fascial spaces in the foot and leg. This study was repeated on a fresh limb hardened in formaline and on another freezed in ice, on the same lines as *Kanavel* had done in his study of the infections of the hand. The spaces, already described by *Manuel Grodinsky* (1929), were identified and followed from section to section along with the following additional spaces described below:

The spaces identified are—

1. *The dorsal subcutaneous space*:—It is an extensive space on the dorsum of the foot and lies deep to the subcutaneous fascia and superficial to the deep fascia. This space is continuous with the subcutaneous space of the leg above and that around the toes distally. It is limited on the medical aspect where the subcutaneous tissue is bound down to the medical border of the phalanges of the great toe, the first metatarsal bone and the tarsal bones behind it, all along the inner margin of the foot. Similarly on the lateral aspect the space

is limited to where the subcutaneous tissue is bound down to the lateral margin of the phalanges of the little toe, the fifth meta-tarsal bone and the tarsal bones behind it all along the outer margin of the foot. (See under injection experiments below).

2. *The dorsal subaponeurotic space :*—It is not so extensive a space as the previous one. It lies deep to the fascia and superficial to another 3rd layer of fascia that stretches across the tarsals and metatarsals and is adherent to them all along their lengths. The deep or aponeurotic layer of fascia permits tunnelling by the extensor tendons as they course down the foot. Fine areolar tissue binds down the aponeurotic layer of fascia to the third deeper layer of the fascia along the length of the metatarsals and on either side of the extensor tendons. Thus the subaponeurotic space is subdivided into a number of longitudinal compartments. The areolar tissue that effects this division is not firm and can be teased out fairly easily.

In the digits the subaponeurotic space is confined to the under surface of the extensor tendons and is obliterated where the extensor expansion is adherent to the articular capsule of the metatarso-phalangeal joint. The deep fascia is adherent to the periosteum underneath along the lateral border of the extensor tendons thus limiting the space laterally.

These details are not found in the literature and experiments will be done to visualise these spaces by radiography.

3. *Median plantar spaces* (four in number), M-I M-II, M-III and M-IV. M-I is deep to the central part of the plantar aponeurosis and lying over the flexor digitorum brevis. M-II is between the flexor digitorum brevis and the flexor digitorum longus and accessorius, and overlies the intertarsal and metatarsal ligaments, the long and short plantar ligaments, the adductor hallucis obliqus muscle and the dorsal and plantar interossei. M-IV is deep to the adductor hallucis obliqus, overlying the interosseous muscles along its course.

4. *Lumbrical spaces :*—One lumbrical space is found in connection with each lumbrical muscle. It is all round the muscle and passes distally around the lumbrical tendon and its synovial sheath. As the tendon joins the extensor expansion this space merges with the digital subcutaneous space. Thus the lumbrical space allows a free passage for infection to spread from the M-II space (from where the lumbrical arises) to the dorsal subcutaneous space and vice versa.

5. *Medical plantar space :*—This space is on the medical part of the sole of the foot. It is deep to the abductor hallucis muscle and lies between it and its investing layer of fascia. The proximal part of the space overlies the tibial vessels and nerves and the flexor tendons as they pass into the foot and is separated from them by the layer of fascia which forms its deep boundary.

6. *Lateral plantar space :*—It is another space in the outer part of the sole of the foot. It is deep to the abductor digiti quinti and lies between it and the fascia lining it. The long peroneous tendon with its synovial and fibrous sheaths lies deep to this space.

7. *Medial leg space :*—This space is in association with the flexor tendons that pass behind the medical malleolus. It is deep to the medical plantar space in the sole and ascends up the leg where it lies between the flexor muscles and the gastrocnemiussoleus group. The posterior tibial vessels and nerve pass through this space. Due to the passage of the flexor digitorum longus tendon

and the tibial vessels and nerve through the medial plantar septum into the central part of the foot infection can track from the medical leg space into M-II and M-III and vice versa.

8. *The lateral leg space :*—It is met in connection with the peroneous tendons as they pass from behind the lateral malleolus into the outer part of the sole. This space is deep to the lateral plantar space in the foot and ascends up in the lateral compartment of the leg deep to the investing layer of deep fascia and posterolateral to the fibular septum. Infection can track up from M-III into this space and vice versa.

Note :— A study of these spaces again in detail was necessary to get a clear understanding of the spread of infection. As a result of this study in detail, the peculiar disposition of the subaponeurotic space with longitudinal compartments has come to light. More experimental study will be undertaken by injecting, radio-opaque fluid material and radiography to substantiate the observation made. Clinical study also will be undertaken.

The following additional spaces have also been identified during the present study :—

1. *The medial plantar space II :*—This is a space that lies deep to the flexor hallucis brevis muscle, between it and the fascia of the muscle. It lies on the body of the first meta-tarsal bone.

2. *The lateral plantar space II :*—This space is deep to the flexor digiti quinti brevis muscle overlying the fifth metatarsal bone. It lies between the muscle and its fascia. It is not so distinct as the previous one.

3. *Web spaces :*—These are limited areas of subcutaneous tissue that lie in the webs of the toes. They are more prominent on the plantar aspect and are continuous with the subcutaneous space at the roots of the digits. The digital vessels and nerves pass through it.

Injection experiments.

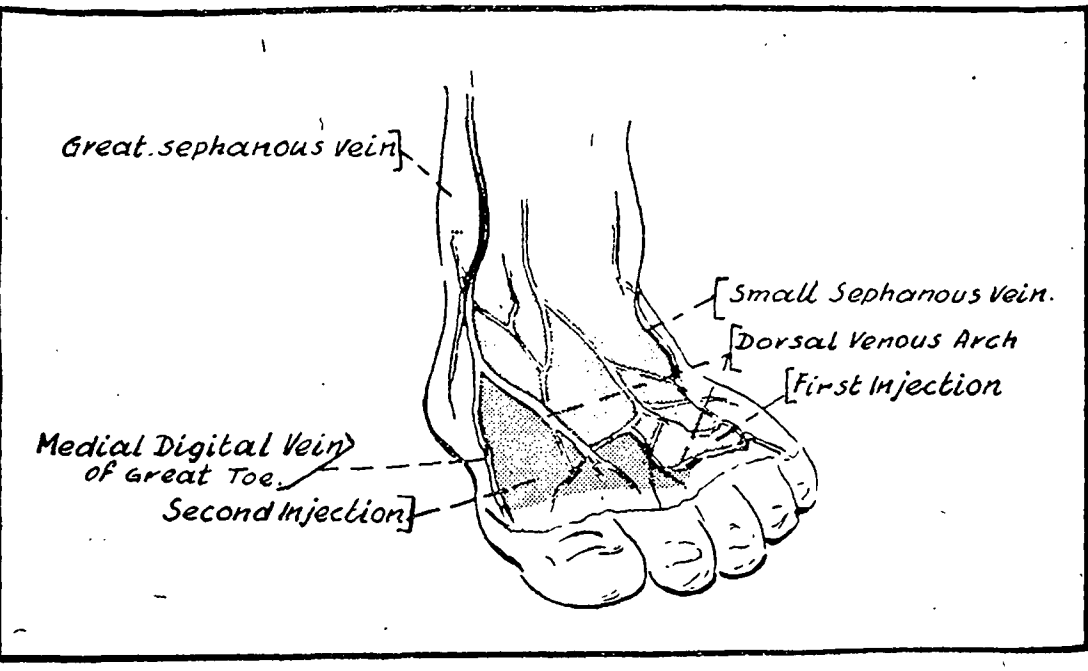
A. *Injection of plaster of paris—glycerine mixture into fascial spaces :*—

Plaster of paris-glycerine mixture was injected with a Leuer Syringe using a wide bore needle into various fascial spaces and the following points noted in connection with the work done so far.

1. *Dorsal subcutaneous space :*—Plaster of paris-glycerine mixture was injected into the dorsal subcutaneous space in the 4th inter-metatarsal region about ½ inch proximal to the head of the 4th metatarsal bone. The space was noticed to fill up from below upwards as the mixture was getting into it. More than 100 cc. of the material was pushed in before resistance was met.

The bulging showed itself on the dorsum of the foot except for a triangular area over the medial part of the forefoot. The medial boundary of the unfilled triangular area was along the length of the distal half of the shaft of the 1st metatarsal bone. Its oblique outer boundary stretched from the midpoint of the shaft of the 1st metatarsal to the head of the 4th metatarsal bone and

the base stretched from the head of the 1st to the head of the 4th metatarsal. (See diagram).



Later, the syringe needle was introduced into the dorsal subcutaneous space in the 1st inter-metatarsal region with a view to inject into the unfilled triangular area and the plaster of paris-glycerine mixture injected into it. That part of the dorsal subcutaneous space that did not fill up during the previous injection now filled up. In both cases material showed a tendency to fill up the space in a proximal direction and not distalwards.

One day was allowed for the plaster to set. On dissection it was found that the dorsal venous arch marked the line of demarkation between the two swellings produced by the injection experiments described above. The small sephanous vein prevented the spread of the material to the outer margin of the dorsum of the foot and the great sephanous vein limited the spread similarly on the medial side (*see* diagram). Thus the medial and lateralmost parts of the dorsal subcutaneous space were left unfilled with the injection material. There was dense areolar tissue around the bigger veins of the dorsum of the foot—the great and small sephanous veins, the dorsal venous arch and the medial digital vein of great toe; and it was this tissue that bound the superficial to the deep fascia along the veins thus limiting the spread of the material beyond the vessels.

This experiment was repeated on another limb and similar observations noted. It is, therefore, obvious that the dorsal subcutaneous space though appears to be a single continuous one from the study of the cross section anatomy, is in fact divided into compartments, depending on the disposition of the dorsal venous arch, the great and small sephanous veins, and the medial digital vein of the great toe.

These details are not found in the literature.

This injection experiment will be repeated on some more limbs.

2. *Web spaces :*—There are four spaces one in connection with each web between the toes of the foot. The boundaries are described below :—

Proximally—plantar aspect—superficial transverse metatarsal ligament with the skin bound down to it.

Distally—the fold of the skin in the web.

Laterally—the skin is attached to the peritendinous digital slips of the plantar aponeurosis as they form a diffuse mesh at the root of the toe, thus forming the outer margins of the space.

Plantar aspect—the skin.

On the dorsal aspect—the web space becomes continuous with the dorsal subcutaneous space.

Each space is a small potential one and is occupied by a few granules of superficial fascia. The digital vessels and nerves pass through the lateral part of the space. Each space is continuous with the subcutaneous space of the digits on either side.

The exact relationship of the lumbrical tunnels with the web spaces has to be established by further experimental study, by injecting the tunnels with radio-opaque fluids and taking radiograms, and afterwards by careful dissection.

Half a c.c. of plaster of paris-glycerine mixture is sufficient to fill the space and resistance is offered when a little more is tried to be pushed in. When resistance is overcome by forcible injection it always spreads both proximally and distally. Distally it spreads along the toes on either side into the digital subcutaneous space. Then in each toe it tries to go round the toe to its opposite side. Thus by the retrograde route the web space adjacent to the primary one can be filled up. Proximally it enters plantar interdigital subcutaneous space traversing underneath the superficial transverse metatarsal ligament. It requires about 4 cc. of the injection material to fill this web space and the plantar interdigital subcutaneous space before resistance is again offered.

In one case more material was injected overcoming the resistance for the second time. On dissection a small part of the plaster of paris was found beneath the skin in the proximal part of the plantar interdigital subcutaneous space, whereas the rest of it was to be found beneath the subcutaneous fatty tissue. This point corresponded to where the central part of the plantar aponeurosis divided into digital slips, thus indicating that this is the weak point where inflammatory fluid collected in the plantar interdigital subcutaneous space might point and try to burst through the skin. Further, the dissection revealed that the material extended into the M-I space along the digital nerve that passed out from the M-I space into the interdigital subcutaneous space.

Radiograms taken after injecting plaster of paris-glycerine paste into the web spaces revealed the spaces and the spread of the material into the plantar interdigital subcutaneous space, around the roots of the toes, and in one case into the M-I space.

Thus, it can be concluded that infections starting in the web space can spread up into the neighbouring web spaces or up the dorsum of the foot into the dorsal subcutaneous space or in the sole proximally into the inter digital subcutaneous space and even into the M-I space.

3. The medial plantar space was filled with plaster of paris-glycerine paste, and an x-ray taken defined its extent clearly. Later it was dissected out entire and the space defined properly.

4. The lateral plantar space-II was filled with injection material and x-ray taken. The radiogram showed the material confined to the lie of the flexor digiti quinti muscle from the base of the fifth metatarsal bone to the base of the proximal phalanx of the fifth digit.

5. Further study is continued. All these details are not given in the literature.

B. *Injection of gelatine and India ink into tendon sheaths—*

The following points have been noted on the work done so far:—

1. *Digital plantar flexor sheaths:*—Their normal limits were defined. They burst at their proximal limits, the material spreading proximally into the M-I space.

2. The sheath of the flexor digitorum longus sometimes communicates with that of the flexor hallucis longus at the point of their crossing.

3. The sheaths of the hallucis longus, digitorum longus and the tibialis posticus tendons all give way at their proximal ends when injected under pressure. The material spreads up the muscles along their myecelial sheaths.

4. Injection into the extensor digitorum longus, the extensor hallucis longus and the tibialis anterior defined their extent and capacity. The material bursts at either end when injected under pressure. Proximally it spreads up along the muscles under their myecelial sheaths. Distally it is localised in the dorsal subaponeurotic space. Manometric readings of the pressure at which these synovial sheaths burst on injection will be taken and further study continued.

5 (a) Injection into the peroneal tendon sheaths reveals their extent. The common sheath at the proximal end bursts with the result that the material spreads up beneath the myecelial sheaths of the muscles. The sheath of the peroneous brevis bursts distally and the material is localised at the point of insertion of the tendon.

(b) The peroneous longus tendon has two separate synovial sheaths, one around it in the sole of the foot and one during its course behind the lateral malleolus. These two often communicate with each other.

(c) The plantar sheath of the peroneous longus bursts at its medial end to spread into M-III and M-IV spaces.

6. Further injection work into the sheaths is being carried out.

Intensive clinical study has been undertaken of all cases of foot injections seeking advice both in the in and out patients.

29. Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

(I) Experimental hepatic cirrhosis.

During the period under review, progress has been made in the experimental work to study the effect of continued feeding of diets containing varying

levels of protein and deficient in the vitamins of the B_2 complex on the histological and biochemical changes in the liver of rats. An outline of the experimental procedure has been previously reported. A short summary of the gross chemical changes in the liver and the histological appearances of the liver in the different groups of animals is recorded below. The results are based on the examination of 180 animals fed on different diets.

(i) *B_2 complex deficient diet with different levels of protein in diet.*

As already reported, casein was the main source of protein and the basal diets contained varying levels of the protein (18, 10, 8 and 5 per cent of casein respectively). Animals in this group were deprived of all the vitamins of the B_2 complex. The following changes in the weight of the liver and in the composition of water, fat and protein in the liver were observed in the different groups.

In general liver weight of the animals varied inversely to the level of casein in the diet, i.e. as the percentage of casein in the diet was decreased, the average weight of the liver per 100 grammes of body weight was increased. In animals receiving 5 per cent casein in their diets the results, however, were not consistent. With the exception of a few rats which showed liver weight of about 10 to 11 grammes per 100 grammes of body weight (Normal Range 3·8 - 4·5 grammes per 100 grammes of body weight), the average liver weight did not show the same relationship to body weight as that observed in the other groups.

The estimation of water, fat and protein contents of the liver showed that this increase in the weight of the liver in the low protein groups was not due to an increase in any single constituent. The amounts of water, fat and protein in liver showed an increase which was also inversely proportional to the level of casein in the diet i.e. when the percentage of casein in the diet was low, the liver on an average contained more water, fat and protein than the liver of animals receiving higher levels of casein in the diet.

However, as mentioned above, at the lowest intake of protein, i.e. at 5 per cent level of casein, the increase both in the liver weight and in the amounts of water, fat and protein in the liver was not similarly proportional. This is mainly due to the low food intake, consequent gradual fall in body weight and the short span of life when the level of protein in the basal diet was reduced to 5 per cent.

Histological examination of the liver in the animals of the various groups corroborated the chemical findings. Varying degrees of fatty change was present in the different groups. The extent of fatty change in the liver was inversely proportional to the level of protein in the diet and was higher in the 8 and 10 per cent groups than in the 18 per cent group. In the 5 per cent protein group, however, it was found to be much lower than in any of the other groups. Although massive necrosis of the hepatic parenchyma was not encountered in any instance, necrosis of the parenchymal cells of the liver appeared to be on the increase as the level of protein in the diet fell from 18 to 5 per cent. Occasional patchy fibrosis was found in the groups receiving 10 and 8 per cent level of protein in their diets. In the 18 per cent protein group it was altogether absent.

(ii) *Control diet.*

In contrast to the above, in the animals which received the control diets, there was practically no increase in the liver weight. The composition of these

diets was similar to the above and likewise the level of protein varied from 18, 10, 8 and 5 per cent respectively; the only difference being that each animal received in addition 0·5 gramme of dried brewer's yeast per day to supply the various factors of the vitamin B_2 Complex. On 5 per cent casein diet, however, the average liver weight of the animals was slightly higher than the other groups. Similarly no significant changes were noticed in the amounts of water, fat, protein and glycogen in the liver from different groups except in the 5 per cent casein group which showed slight increase in the water and fat content.

Histological examination of liver showed that in the 18 per cent protein group fatty change was absent, whereas in the 10, 8 and 5 per cent casein groups it tended to increase gradually as the protein level in the diet was reduced. Necrosis of the parenchymal cells of the liver was not a prominent feature and was only present in the very low protein groups. Occasional patchy fibrosis was found in the 10 and 5 per cent groups.

It seems evident from the above that intake of protein and vitamins of the B_2 Complex have an important bearing on the chemical composition and histological structure of the liver. In general low protein diets and diets deficient in vitamins of the B_2 Complex have a tendency to accumulating fat in the liver.

At the suggestion of Dr. Neuberger, a member of the staff of the Medical Research Council, London, who visited the Haffkine Institute last winter, the calculations for water, fat and protein content were based in terms of grammes in liver per 100 grammes of body weight. The results obtained in this investigation are in general in agreement with those reported recently by Himsworth and his colleagues.

(iii) *Effect of restricted intake of protein in an otherwise adequate diet on the Biochemical and histological changes in the liver of rats.*

In the previous experiments the different diets were fed to rats *ad libitum*. It was found that due to variation in the total amount of food consumed, there was considerable difference in the intake of protein in the same group of animals. It was, therefore, considered desirable to limit the intake of protein at particular levels. Four groups of rats were accordingly given purified synthetic diets in such quantities that rats from the first group received a maximum of 1·2 grammes of casein per rat per day, while those in the second, third and fourth groups received 0·6, 0·4 and 0·2 gramme of casein per rat per day. Each rat was given in addition 0·5 gramme of yeast per day. This experiment was continued for 150 days after which one animal from each group was killed at regular intervals.

A record of the daily food intake of the animals as also their weekly body weight was kept. It was found that the growth of the animals in this group was proportional to the intake of protein. In addition to the chemical and histological examination of the liver, total plasma protein was also estimated. These investigations are now in progress.

(II) Clinical investigations on cirrhosis of the liver.

Arrangements have been completed for carrying out the clinical investigations on cirrhosis cases in the city hospitals and work on this aspect has just been commenced.

An advanced case of toxic cirrhosis of the liver in which a partial post-mortem was available was studied in detail.

30. Enquiry on cirrhosis in animals and its bearing on the aetiology of infantile biliary cirrhosis under Dr. T. Bhaskara Menon at the Andhra Medical College, Vizagapatam.

Experimental studies on sucklings and young animals (puppies and rabbits) have been carried out and are being continued with regard to an excess of fat in the diet in addition to mother's milk, to determine the nature of the fat and a possible toxic factor that is responsible for fat-loading and the subsequent cirrhosis. Three sets of experiments have been carried out so far, two sets with puppies and one set with young rabbits. A fourth set of experiments with puppies has just been started.

In the first set of experiments pure castor oil (3 c.c. per kilogram weight) was administered and the effect of large doses studied. In this set of experiments the gastro-intestinal effect of the ricinoleic acid was so marked that there was little absorption, liver lesions were ill defined and the animals died of inanition due to chronic diarrhoea. In two animals secondary infection (one a broncho-pneumonia and the other a portal pyemia) were responsible for the death of the animals.

In the second set of experiments pure castor oil (0·3 c.c. per kg. wt. gradually increased to 0·6 c.c. per kg. wt.) was administered and the effect of small daily doses was studied. In this set of experiments fat-loading of the liver became noticeable when the feeding was continued from 90 to 100 days; but no cirrhotic lesions have yet been encountered, but the feeding is being continued.

In a third set of experiments pure olive oil (0·5 c.c. per kg. wt.) was administered to young rabbits and the effects of the bland oil which has no irritant effect on the gastro-intestinal tract are being studied. So far the experiments indicate that the fat-loading is well marked. Cirrhotic lesions have not yet developed, but the experiment is being continued.

The fourth set of experiments with crude castor oil (as sold in the bazaar and usually administered to the children of the lower middle classes) to determine a possible 'ricin' effect has been just started. The sample of castor oil is being carefully analysed chemically for the ricin content.

The fifth set of experiments which have not yet been started are with the essential oils and spices that form the usual ingredient of the diets in India. They are designed to determine if the change in the diet at the sixth month that usually takes place in India children has any possible aetiological significance.

31. Enquiry on infantile cirrhosis under Dr. P. Kutumbiah at the Stanley Medical College, Madras.

Work was carried out on the following lines:—Standardisation of the methods for estimation of (i) choline, (ii) methionine, (iii) cystine, in human milk.

The colorimetric method using photo-electric colorimeter, according to the procedure of Engel (J.B.C. 1942, Vol. 144) is being used. A sample of the milk is evaporated to as small a bulk as possible in vacuum. It is extracted with methanol under reflux for 20 to 24 hours. The extract filtered through asbestos

pad and the filtrate evaporated to near dryness under reduced pressure. The residue is treated with saturated aqueous baryta for 2 hours at 100° C. After cooling, the hydrolysate is neutralised with acetic acid and filtered. The filtrate is treated with 2·% solution of ammonium reinckate in methanol. The precipitate of Chloine reinckate is filtered, dissolved by acetone on the filter and the pink solution is estimated using the photo-electric colorimeter, previously calibrated with ten solutions of choline chloride of known strength.

Methionine is estimated colorimetrically using the sodium nitro-prusside reaction according to the procedure of Heartly & Sullivan (J.B.C. Vol. 141).

Cystine is estimated colorimetrically using Sullivan's method.

A few samples of human milk, (within the first week of delivery) were analysed. The results are being confirmed by analysis of more samples.

The content of choline, cystine and methionine in human milk will be investigated with respect to (i) varying income groups of the subject; (ii) in subjects with cirrhotic children.

Clinical.

The scope of the clinical part of the investigation was confined to (a) detailed recording of the history of the case with particular reference to the dietetic factor and (b) study of the response of selected cases to treatment with choline chloride over prolonged periods.

Twenty-one cases of the disease were encountered in the course of the investigation and 5 of these, which could be depended upon to undergo the complete course of treatment, were subjected to the therapeutic test.

An analysis of the history of the 21 cases revealed, besides other fact, that—

(1) Infantile biliary cirrhosis is not a disease of the wealthy classes but affects the lower middle and poor classes in greater measure.

(2) It is not particularly an affection of the Brahmin community but affects the non-Brahmin and other communities considerably.

(3) No subject of the disease was entirely fed on breast milk alone, or on any proprietary milk preparation; a majority of them (19) were on mixed feed—breast milk and diluted cow's milk—and a small minority (2) were fed on diluted cow's milk alone.

(4) Judging from the evolution of the clinical symptoms there were mainly *two types* of cases—one in which the disease process runs an acute course to a fatal termination in a *few weeks* and the other in which the process is drawn out over *several months and even years*.

Three of the five cases treated with choline chloride died. Two of these belonged to the acute type and expired within a week of starting the treatment. The third case which presented deep icterus and gross enlargement of the liver at the commencement of treatment underwent the treatment for nearly 3 months receiving 3 gs. choline chloride by mouth daily. The treatment seemed to retard the fatal course of the disease and caused an appreciable reduction in the hepatic enlargement; but an acute febrile reaction set in at the close and the patient died of cholaemia.

Two cases which were treated with choline chloride improved. Both these were cases of less than 3 months' duration, with moderate enlargement of the liver, alimentary upset and fever. Ascitis was absent in both and jaundice was absent in one. In the other cases jaundice was intermittent. Treatment with choline chloride for 8 weeks caused an appreciable reduction of the size of the liver and the symptoms disappeared. The patients were however instructed to subsist purely on skimmed milk and fruit during the treatment. Before conclusions are actually drawn from this, it is proposed to treat cases of comparable severity subjected to identical dietetic restrictions on other lines to see if the improvement noticed is not due to the diet alone.

Investigation on these lines is being continued.

32. Dental research unit under Dr. C. D. Marshall Day at the de Montmorency College of Dentistry, Lahore.

The unit was constituted on the first of April, 1946 and for the first 1½ months the services of Dr. K. L. Shourie (Research Officer attached to the unit) were placed at the disposal of the Secretary, Indian Research Fund Association. During this period Dr. Shourie carried out diet and nutritional surveys in rural and urban areas of Ajmer-Merwara Province and submitted a detailed report on the nutrition problem in Ajmer-Merwara Province. He also undertook a dental and parodontal survey in Ajmer-Merwara which revealed a low incidence of dental caries and a very high incidence of parodontal diseases. Dental fluorosis was found to be in endemic form in that area. A paper has been submitted for publication in the I.J.M.R.

During the last week of May 1946, the Director of the Unit, Dr. C. D. Marshall Day proceeded on ex-India leave on grounds of health. During his stay in England he was invited to attend a meeting of the Medical Research Council (Medical Research Liaison Committee for India), where he gave an account of the background of dental research in India. An opportunity was also taken to establish a liaison between the Dental Research Unit in India and the Medical Research Council of Great Britain.

During the year 1946 the following investigations were undertaken in addition to the work above mentioned:

(1) An experiment was conducted in relation to the infantile scurvy and dental dystrophies resulting from antiscorbutic deficiency during lactation. This work was begun previous to our liaison with the Indian Research Fund Association and completed under their auspices. A complete report has been sent for publication in the I.J.M.R. but the results may be briefly summarised :—

Following the preliminary investigation of latent scurvy and its relation to dental defects, and of the effects of antiscorbutic deficiency upon the pregnant organism, this experimental work was undertaken with the object of investigating the effect of relative and absolute hypovitaminosis C upon lactating mothers and their young.

As in the earlier work particular attention was given to the effect on the dental tissues, but the opportunity was also taken to study the reaction to this specific deficiency of the body organism as a whole.

Our results leave no room for doubt that the specific needs of the organism for the antiscorbutic vitamin are equally as great during the period of lactation as during the gestation period.

Growth curves indicate that the average and percentage weight loss during the experimental period of 20 days lactation was inversely proportional to the amount of antiscorbutic received by the mothers. As the only variable was the antiscorbutic potency of the mother's milk it may, therefore, be assumed that vitamin C was secreted into the mother's milk in approximately the same ratio in which it was administered to her by mouth.

Although, as might be expected, there was considerable individual variation, it was found that the average incisor growth per day in both the upper and lower jaw was directly proportional to the amount of antiscorbutic administered. The average growth of the lower incisors was in all cases greater than that of the uppers. The mean average incisor growth per day was, therefore, also proportional to the vitamin C intake.

These remarkably constant results would appear to confirm the observation that the rate of growth of the incisor teeth of guinea pigs is a precise indication of the degree of scurvy. It is therefore reasonable to assume a specific response of the teeth of the presence or absence of the antiscorbutic vitamins.

The incisor growth rate was less in lactating animals than in non-lactating animals of the same weight. This would seem to indicate an intensified effect of a lack of vitamin C during lactation.

It is considered that the figures showing the percentage of total minerals in the bones and teeth are not of particular significance in view of the short experimental lactating period and for other reasons advanced it is thought that over this short period more importance attaches to qualitative changes than to quantitative changes in the mineralization of the bones and teeth.

A study of the photomicrographs from transverse sections through the mandible and incisor teeth clearly demonstrates the profound effect of hypovitaminosis C on the dental tissues and illustrates the importance of an adequate maternal diet during the period of lactation not only for the protection of the mother but also to ensure adequate amounts of vitamin C in the mother's milk for effective protection of the young. Although the mother's diet may be only slightly deficient in vitamin content the antiscorbutic potency of the milk is proportionally reduced, resulting in marked changes in the dental tissues of the young.

These pathological tissue changes run parallel to the other observed phenomena of interference with body growth and tooth growth and occur even in the absence of scurvy symptoms clinically recognisable as such.

It is apparent that the microscopic tooth picture of both the lactating mothers and their young can be altered at will by varying the amounts of antiscorbutic in the mother's diet; further if the vitamin C potency of the mother's milk is insufficient through faulty diet, dental dystrophies and other degenerative changes may be prevented by the addition of suitable amounts of vitamin C as a supplement to the milk of the mother.

The effect of hypovitaminosis C on the jaws and facial bones is very marked. In mature animals the alveolar bone becomes thin and porous and the teeth loose and irregular due to lack of support by the periodontal structures.

During the developmental period similar degenerative changes occur. Radiographs show that the growth of the jaws and alveolar ridges is directly proportional to the vitamin C intake.

During vitamin C deficiency characteristic features of the systemic type of gingivitis and pyorrhoea occur in experimental animals. In this connection it is of interest to note that a very high incidence of gingivitis has been observed in the famine district of Hissar where numerous cases of florid scurvy have been clinically recognised.

In the light of these studies it is considered that much of the degeneration of the oral tissues and bodily ill-health has its origin in specific vitamin deficiencies and that scurvy of the latent or subacute type is much more common than is generally supposed.

In countries such as India where the dietaries of a large proportion of the population in many areas are greatly restricted through ignorance, poverty or climatic conditions the danger of specific deficiencies is very great. Recent investigations undertaken and health measures adopted in the deficient and famine areas of the Punjab have clearly shown the futility of improving the quantity of the diet unless these specific deficiencies are at the same time eliminated.

(2) Age of onset of parodontal disease :—Pyorrhoea is known to be characterised by progressive destruction of the alveolar bone and the periodontal membrane. Radiograms have accordingly been recommended for diagnostic purposes. To ascertain the age of onset of parodontal disease full mouth x-rays of 500 persons of various age groups were taken. X-rays of various groups require a careful comparative study before conclusions can be reached, so the whole series of 5,000 radiograms is at present under detailed study and a report will shortly be published. Present indications are that the onset of parodontal disease, with the accompanying bone absorption, in this country takes place at an early age.

(3) The effect of urban life on gum conditions have been studied on a group of 100 workers of Lahore Corporation and a similar group of additional police constables. It would seem from the preliminary study of the x-rays of these groups that our clinical observations, that the incidence and degree of parodontal disease is higher in urban than in rural groups will be confirmed.

(4) The effect of metabolic distrubances on the aeteology of parodontal disease have been studied on 200 women who were examined clinically. Data have been collected regarding the extent of disease and the number of pregnancies, and are being analysed.

Similar data collected in respect of dental caries do not seem to confirm the concept of a relationship between pregnancy and dental caries. A paper has been prepared and submitted to the Editor, I.J.M.R. for publication.

(5) Investigation has shown that there are a number of endemic fluorosis areas scattered throughout the Punjab and other parts of India and this condition is of considerable public health importance in view of the general systemic effects. Further importance attaches to these findings in view of the recent interest in fluorine as a factor conferring immunity to dental caries and the long-term mass experiments being carried out in relation to the water supply of cities in England and America.

To supplement research already carried out by this unit in endemic areas such as Kasur and Hissar where a very low incidence of dental caries was reported, and to establish a background for further investigation, 300 samples of drinking water were collected from districts in the Punjab province and analysed for the fluorine content. This data is being correlated and will be published shortly.

The effects of the ingestion of an excess of fluorine on gums and periodontal membrane have been studied in a group of 500 school children from an endemic fluorosis area. The results will be compared with data to be collected from certain non-endemic areas.

(6) To discover whether there are any sex differences in the incidence of parodontal disease, 326 girls of a high social status attending Queen Mary College, Lahore were clinically examined. The results show a high incidence of gingivitis in this group. The findings in this and other investigations previously carried out indicate that gingivitis is an early manifestation of the almost universal parodontal disease of adult life.

(7) Experience in working with the established methods of evaluating the intensity of parodontal diseases show that procedures ordinarily employed were not sufficiently definite and exact. Thus a new technique for the estimation of the degree of parodontal disease has been adopted and perfected. This technique will shortly be published along with the findings in groups at present under examination.

33. Enquiry on the relationship between the measurements of vertebrae and body size of Indians under Dr. R. G. Ginde at the Seth G. S. Medical College, Bombay.

The work was started in August 1946. Measurements of the horizontal interpediculate distances of vertebrae from x-ray plates of the spines from the records of the K.E.M. Hospital were taken. About 600 x-rays have been scrutinised so far. These x-rays include both normal and diseased spines of patients of both sexes over the age of 12. In the case of diseased spines, the measurements of the diseased vertebrae were not taken. Table I shows the result of these measurements as compared with those of Elseberg.

The average figures on comparison show a definite difference in the measurements along the whole length of the spinal column.

In addition to these 62 students of the college were examined, their heights, weights etc., were taken and the spines of 39 of them have so far been x-rayed using a uniform technique. The interpediculate distances from the x-rays have been measured and further work is in progress.

Besides this, vertebrae from the same skeleton in 8 cases were also measured for the interpediculate distances and it appears that the figures almost approximate to those from x-ray plates of the spines. Table II shows the measurements from the skeletons.

If this is confirmed by further work it would be conclusive that the x-ray measurements give almost the actual size of the vertebral canal especially in the thoracic lumbar region.

TABLE I.

Vertebrae	2	4	4	5	6	7	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5
No. of X-rays	36	74	107	116	126	137	167	180	183	171	155	150	142	171	244	309	380	403	419	409	406	389	357
Average H.I.D. in m.m.	26·8	26·9	26·8	27·1	27·8	27·1	22·7	19·5	18·2	17·1	16·4	16·8	17·2	17·4	17·6	18	18·6	21·9	23	23·3	24·2	25·4	28·3
Elseberg's figures	30	30	32	32	32	31	27	23	22	20	20	20	20	20	22	21	23	26	28	29	30	31	33

TABLE II.

No. of skeletons	6	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	7	8	8	8
Spines	2	3	4	5	6	7	1	2	3	4	5	6	7	8	9	10	11	12	1	2
Average H.I.D. in m.m.	22·3	22·3	23·1	23.9	24·4	23·5	20·5	17·7	16·3	15·9	15·5	15·4	15·4	15·5	16·1	16·1	16·7	18·9	20·3	20·4

No. of skeletons ..	8	8	8
Spines ..	3	4	5
Average H.I.D in. m.m. ..	21	21·3	24·5

34. Enquiry into causes of blindness under Captain E. J. Somerset, I.M.S., at the Medical College, Calcutta.

Work was started on April 15th, 1946 and up to 31st December, 1946, 1,400 cases have been seen and analysed.

Table 1, shows what enquiry and investigation was made in each case. The items in Table 1 have then been transferred to special cards which have been analysed by the Powers-Samas (Sorting) Accounting Machine under Professor Chandrasekar at the All-India Institute of Hygiene and Public Health. A thousand cases have been thus analysed but it is not proposed to draw any detailed conclusions from these figures. This will be done when another two or three thousand cases have been recorded. It would appear that blindness is preventable in at least 38% of cases.

Tables 2, 3, 4, 5, 6 and 7 give an idea of some of the ways in which cases are being analysed statistically.

TABLE 1.

Enquiry into the causes of blindness.

Medical College Hospitals, Calcutta.

1–4.	Serial No.								
	Name					Calcutta Address			
5.	Usual Residence :	Bengal	Bihar	Orissa	U.P.	Assam	Other provinces		
		1	2	3	4	5	6	(specify)	
6–7.	District for Bengalees.		(				)		
8–9.	District for Biharees.		(				)		
10.	Whether came to Calcutta for treatment.					Yes	No		
						1	2		
11.	Age.	0–1	1–4	5–15	16–29	30–39	40–49	50–59	60 & over.
	(Completed years).	1	2	3	4	5	6	7	8
12.	Sex			Male			Female		
				1			2		
13.	Religion.	Hindu		Moslem		Christian	Others (specify)		
		1		2		3	4		
14.	Diet	Veg.		Veg. & Fish.		Veg. Fish & Meat.			
		1		2		3			
15.	Milk for children.	Nil		½ pau		½-1 pau	Over 1 pau		
	(Daily intake)	1"		2		3	4		
16.	Occupation (prior to eye trouble)			(			)		
17.	Which eye affected	R. Eye.	L. Eye.	Both.	Which one first.	Right.	Left.	Simultaneously.	
		1	2	3		4	5	6	
Right Eye.									
18.	Duration of disease.								
19.	Duration of blindness.								

20. Clinical findings & diagnosis.

21.	Main group :	Congenital & undetermined conditions.	Infectious & Toxic Exogenous.	Infectious & Toxic Endogenous.	Traumatic & chemico toxic.	General disease.	Unclassified.
		1	2	3	4	5	6
22–23.	Sub-group.						
24–25.	Detailed group.						

26.	Previous treatment.	Nil.	Quackery.	Qualified.	Others.
		1	2	3	4
27.	Why no treatment.	Ignorance.	Lack of medical facilities.		Expense.
		1	2		3
28.	Could proper treatment have improved sight.			Yes. 1	No 2
	Could it have improved earning capacity.			Yes. 3	No 4
29.	Prognosis.	Curable.	Partially curable.		Incurable.
		1	2		3

Left eye. Ditto.

BLINDNESS ENQUIRY.

Medical College Hospitals, Calcutta.

TABLE 2. *Age incidence.*

Age	0–1	1–4	5–15	16–29	30–39	40–49	50–59	60 & over	N.K.	Total
Sex	1	2	3	4	5	6	7	8	—	—
Male	12	17	75	136	123	105	157	129	—	754
Female	2	9	30	21	22	43	61	58	—	246
Total	14	26	105	157	145	148	218	187	—	1000
Percentage	1·4	2·6	10·5	15·7	14·5	14·8	21·8	18·7		

TABLE 3. *Eye affected.*

R. Eye.	L. Eye.	Both Eyes.	N. K.	Total.
(1)	(2)	(3)	—	—
362	319	319	—	1000

TABLE 4.

Whether came to Calcutta for treatment.

Yes	No	N.K.	Total
(1)	(2)	—	—
707	293		1000

Blindness Enquiry.

Medical College Hospitals, Calcutta.

Table 5.

Single eye affected	Non-industrial injury														Traumatic: Industrial injury											
Right or left																										
Clinical finding & diagnosis	Corneal opacity (1)		Adherent Lucoma (2)		Staphyloma (3)		Pthisis bulbi (4)		Others (5)		N.K.		Total		Corneal opacity (1)		Adherent Lucoma (2)		Staphyloma (3)		Pthisis bulbi (4)		Others (5)		N.K.		Total	
Previous treatment	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.	R.	L.
Nil (1)	—	—	1	1	—	—	1	2	1	1	—	—	3	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Quackery (2)	—	—	—	1	1	1	—	1	1	2	—	—	2	5	—	—	—	—	—	—	—	—	—	1	—	—	—	1
Qualified (3)	—	1	5	2	1	3	6	9	12	6	—	—	24	21	2	1	3	3	—	—	—	3	8	4	—	—	13	11
Others (4)	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
N.K.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total.	—	1	6	4	2	5	7	12	14	9	—	—	29	31	2	1	3	3	—	—	—	3	8	5	—	—	13	12
Prognosis																												
Curable (1)	—	—	—	—	—	—	—	—	5	2	—	—	5	2	—	—	—		—	—	—	—	2	1	—	—	2	1
Partially curable (2)	—	1	6	1	—	1	—	—	6	3	—	—	12	6	2	—	2	3	—	—	—	—	4	3	—	—	8	6
Incurable (3)	—	—	—	3	2	4	7	12	3	4	—	—	12	23	—	1	1	—	—	—	—	3	2	1	—	—	3	5
N.K.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	—	1	6	4	2	5	7	12	14	9	—	—	29	31	2	1	3	3	—	—	—	3	8	5	—	—	13	12

Blindness Enquiry.

Medical College Hospitals, Calcutta.

Table 6.

Cause of blindness.	Right Eye		Left Eye		Both Eyes		Both Eyes same cause.	
	Number	Percentage of Total	Number	Percentage of Total	Number	Percentage of Total	Number	Percentage of Total
Congenital & Undetermined	159	46·69	130	40·75	299	43·91	156	53·24
Exogenous Infection	85	23·48	90	28·21	175	25·70	51	17·41
Endogenous Infection	61	16·85	47	14·73	108	15·86	57	19·45
Traumatic & Chemico Toxic	42	11·60	43	13·48	85	12·48	4	1·37
Systemic Diseases	5	1·38	9	2·82	14	2·06	25	8·53
Total	362	100·00	319	99·99	681	100·01	293	100·00

Table 7.

Both Eyes.

Right Eye & Left Eye	Congenital & Undetermined	Exogenous Infection	Endogenous Infection	Traumatic & Chemico Toxic	Systemic Diseases	Total
Congenital & Undetermined	156	8	2	—	—	166
Exogenous Infection	1	51	5	—	—	57
Endogenous Infection	1	6	57	—	—	64
Traumatic & Chemico Toxic	2	1	—	4	—	7
Systemic Diseases	—	—	—	—	25	25
Total	160	66	64	4	25	319

PHARMACOLOGY

35. Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S. (R) at the Drug Research Laboratory, Jammu, Kashmir.

Chrysanthemum cinerarifolium Vis.

Studies were undertaken to evaluate the effect of different methods of drying, and of the age of the plantations on the pyrethrin content of pyrethrum flower heads. A large number of samples from the various localities where pyrethrum is cultivated in Kashmir were collected.

Methods of drying :—Samples of pyrethrum flowers dried under different conditions were analysed for their pyrethrin content. From a perusal of the results obtained, it was observed that the total pyrethrin content was highest in the case of flowers which were dried for three successive days under the sun, the drying being then completed in the shade. It was lowest in the case of flowers dried under the sun alone for seven days. Exposure to the sun for four days was sufficient to dry the flowers and further exposure resulted in deterioration of pyrethrins.

Effect of age :—Samples of pyrethrum flowers from plantations of ages ranging from one year to seven years were collected. Results of analyses indicate that there is a gradual decrease in the pyrethrin content of the flower heads with increasing age of the plant. This decrease becomes appreciable after the third or fourth year of the life of the plant.

Colchicum luteum Baker.

Colchicum corms from various localities in Kashmir were collected to study the difference in the colchicine content of the corms collected at two stages i.e., when the plant is in the flowering stage and when the flowers have been shed.

It was observed on analysis that there was practically no difference in the colchicine contents of the corms collected at the two stages mentioned above.

Atropa accuminata.

Experiments to investigate the proper time of collection of belladonna leaves, i.e. when the alkaloidal contents are at a maximum, were undertaken. Samples of leaves at different stages of development of the plant were collected from Sindh and Gulmarg Ranges. It was observed on analysis of these samples that the best time for harvesting of the Indian belladonna leaves is in early August when the plant starts flowering. As the season advances the alkaloidal content decreases and after the flowers are shed there is a marked decrease in the alkaloidal content.

Rhamnus virgatus.

The fruit of this tree is extensively used for purgative purposes in indigenous medicine. In order to study the possibility of the bark of this tree being employed as a substitute for the official cascara sagrada bark, a sample of rhamnus virgatus bark was procured locally. Relevant tests prescribed by the B.P. and U.S.P. were conducted and it was observed that though the active principles are present the bark does not come up to the official standard.

Essential oil bearing plants.

Work on essential oil bearing plants growing wild in the Kashmir Valley was continued during the year and the following plants were studied.

1. *Acorus calamus* (*Vern. Via*) : The plant is widely used in indigenous medicine in the treatment of asthma, dysentery and diarrhoea as an emetic, nauseating and carminative agent. It grows wild in Kashmir valley in damp marshy areas. The fresh and unpeeled rhizomes of the plant were steam distilled and a brown yellow oil was obtained.

Yield of the oil	3·1 per cent.
Specific gravity	1·081 at 15°C.
Refractive index	1·5475 at 20°C.

2. *Cinnamon tamala* :—(Vern. Dalchini, Tejpat). The cinnamon tamala trees grow wild in the Rajouri forests of Jammu and Kashmir State. Both the bark and the leaves possess a cinnamon like odour and are used locally in place of the cinnamon bark. Both the leaves and bark were steam distilled and a yellow oil with cinnamon like odour was obtained.

Yield of the oil from bark	0·05 per cent.
Yield of the oil from bark	1·2 per cent.
Specific gravity	1·023 at 15°C.
Refractive index	1·4775 at 20°C.

3. *Hyssopus officinalis*. (Vern. Zufah-Yabis) :—The leaves of this plant were much used in indigenous medicine as stimulant, stomachic, expectorant, diaphoratic and emmenagogue. The plant is exotic and has been successfully cultivated in the forest nursery at Baramulla. The fresh and dry herb was steam-distilled and a brownish yellow oil with pleasant odour was obtained.

Yield of the oil	0·7 per cent.
Specific gravity	0·937 at 15°C.
Refractive index	1·4775 at 20°C.

4. *Angelica glauca* (Vern. Chora) :—This plant grows in abundance throughout the Kashmir Valley and the roots of the plant are much used by the inhabitants as a spice. In the indigenous medicine the plant is used in the treatment of dysentery, constipation and as stimulant and cordial tonic. The dry roots were steam distilled and a brown oil was obtained.

Yield of the oil	1·2 per cent.
Specific gravity	1·0275 at 15°C.
Refractive index	1·4675 at 20°C.

5. *Elsholtzia densa* (Vern. Pudina) :—This is an annual herb found growing wild in Kashmir valley. The whole dry herb was steam distilled and brown oil was obtained.

Yield of the oil	0·98 per cent.
Specific gravity	0·9697 at 15°C.
Refractive index	1·4675 at 20°C.

Pharmacological investigations.

1. *Lochnera pussila* :—The chemical investigation of the plant was completed last year. The plant was found to possess an alkaloid to the extent of 0·22 per cent. No other active principles were discovered.

The physiological action of the plant was investigated. Intact animals were fed with varying doses of the crude drug as well as with a decocation prepared from it. It appeared to be quite inert.

On intravenous administration of 1·0 per cent solution of the total alkaloids to urethranized animals, no appreciable effects could be observed either on the blood pressure or respiration even with such doses as 10 to 20 mg per kilo body weight. As a result of these experiments it is concluded that the alkaloid is physiologically inactive.

Digitalis purpurea :—Deterioration in the biological activity of digitalis preparations in tropical climates has been established. The deterioration of potency in case of tincture of digitalis may be as much as 50 per cent within a few months.

A number of samples of powdered digitalis leaf were stored under natural conditions and in cold room. On examination deterioration in the potency of the powder was found to be not more than 8 to 10 per cent after storage for one year under these conditions. Since the number of samples examined was small further work is being done with a large number.

Pseudo-ephedrine.

The Director-General, Indian Medical Service, forwarded the alkaloid pseudo-ephedrine for a detailed investigation of its pharmacological action. The action of the alkaloid on heart was investigated by Chopra and co-workers and a preliminary note was published. Further work of a confirmatory nature was undertaken. A large number of experiments were conducted on cats and rabbits. The administration of 2 mg of pseudo-ephedrine intravenously produced a rise of blood pressure both in the intact and decerebrate animals. This rise of blood pressure could be seen even after administration of atropine and nicotine in doses sufficient to paralyse the vagus and sympathetic respectively. It was shown that there was definite increase both in force and frequency of the beat of ventricles and auricles. Detailed investigation of the alkaloid on other systems is in progress.

MATERNITY & CHILD WELFARE.

36. Enquiry into the bearing of premature and immature births on infant mortality under Dr. B. C. Das Gupta, Executive Health Officer, Bombay Municipality, Bombay.

Preliminary.

The enquiry commenced on the 1st September 1946 and the material is being obtained from 14 dependable institutions in different areas of the city, thus giving a representative sample of cases which embrace all areas and include mothers from all communities and all strata of society. This is an interim report and will serve only as a fact finding one.

Method of enquiry.

For the purposes of the enquiry any living infant weighing less than 5 lbs. has been treated as premature or immature, according to the period of pregnancy, as far as it was possible to ascertain, from the mother and from a study of the pre-natal and natal case forms.

Those classified as premature and immature are deemed so for the following reasons :—

(a) A careful study of the pre-natal and natal case forms wherever these were available.

(b) A careful inquiry into the mother's history.

(c) The height of the fundus and girth of the abdomen which averages—height 11 in.—girth 30 in. (not very reliable).

(d) The anatomical and physical characteristics such as the general appearance of skull, skin, adipose tissue, lanugo, shoulder and trunk proportion, sutures, fontanelles and the cry of the infant.

(e) The length of the infant, circumference of its head, the weight of the placenta and the weight of the infant which averages 17½ in., 12½ in., 14 ozs., and 4 lbs. to 4 lb. 8 oz. respectively (wherever available).

Collection of statistical material with all clinical details from the mother during her stay in the institution. Follow up of the mother in her home in order to study the economic, social, dietetic and housing conditions. Owing to the abnormal conditions prevailing in the city the follow up work has become extremely difficult.

Work done.

The material obtained during the four months, viz., 1st September 1946 to 31st December 1946, collected, sorted out and tabulated in order to determine as far as possible the cause or causes at the root of these premature and immature deaths, both from the medical as well as the social aspects of the problem. This is with a view to enable correct inference being drawn from the study and proper measures being adopted to check and reduce the number. The data obtained will be later subjected to statistical analysis.

Statistics collected.

The total number of premature and immature infants under enquiry was 1,145 and the total number of mothers who gave birth to these infants was 1,102. The 43 extra babies were 41 twins and 1 triplet.

Maternal findings.

(1) Parity of mothers—out of 1,102 mothers 351 were primi-paras, 697 multi-paras; in 54 cases information was not available. This shows that premature and immature births occur twice as often in multi-paras as in primi-paras.

(2) Multiple pregnancies—there were 42 multiple pregnancies of which 41 were twins and 1 triplet.

(3) Age groups of the mothers—the largest number occurred in 20 to 30 age group which is not unusual even for full term infants; it is found that the largest number of full term infants also occur in this age group; this seems to be the most fertile period in the age of a woman.

TABLE I.

Age group.	Number.
Below 20	191
Between 20 and 30 ..	731
Between 30 and 35 ..	73
Between 35 and 40 ..	32
Between 40 and 45 ..	8
Information not available ..	67

(4) Distribution by communities :—

TABLE II.

Community.	Number.
Hindus	769
Mohammedans	140
Jews (including 1 Bagdadi)	17
Indian Christians ..	133
Anglo-Indians	3
Parsees	5
Awaiting ascertainment ..	35

Hindu mothers formed the largest number, being 5 times more than those of other communities. The Hindu community is far larger than the other communities in the city and most of the contributory causes leading to premature and immature births seem to be more common among Hindu mothers than among others.

(5) Type of care received by mothers during pregnancy :—793 mothers attended pre-natal clinics and their visits to the clinics averaged from 1 to 10. About 30% of the mothers had only 1 visit to the clinic. Six were attended by private doctors, 220 had no medical care; in 83 cases information was not available.

(6) Previous obstetric history :—In 30% of the cases the history of previous labour showed a series of premature and still-births, and in some instances, infant deaths in the early months of life.

(7) Health of the mothers during pregnancy :—Only 32% of the mothers were healthy and had no pathological condition present. Among the

pathological conditions present, anaemia accounted for 48% and malnutrition and debility for 20%.

(8) Labour :—840 were cases of normal labour, 56 abnormal, 12 instrumental, and 102 operative. There was artificial interference in 28 cases. Information in 64 cases could not be ascertained.

(9) Puerperum :—Out of 1,102 mothers, 929 had an afebrile and uneventful puerperum, 5 had mild sepsis, 64 had pyrexis lasting for 4 or 5 days, 52 had a day's temperature during the puerperum and in 1 case the puerperum was complicated with typhoid and pneumonia. In 51 cases the information was not available.

(10) Maternal mortality :—The maternal mortality of mothers during their stay in hospitals was 6 and that in the first month after delivery and discharge from hospital was 6 in 304 mothers followed up. The total maternal mortality was 12 or 10·9 per 1,000.

Infant findings.

(a) Distribution by sexes :—The total number of infants 1,145. 521 were males and 566 females; information in case of 58 cases requires to be completed.

(b) Distribution by maturity :—Of the 1,145 infants. 437 were premature and 634 immature; information in 74 cases requires to be completed.

(c) Period of maturity in months :—Table III gives the period of maturity in months.

TABLE III.

Period in months.	Number.
7th month	145
8th month	292
9th month	634
Information not ascertainable	74

(d) Grouping of infants by weight :—

TABLE IV.

Weight.					Number.
lbs.	ozs.	to lbs.	ozs.	..	
1	8	2	8	..	53
2	9	3	8	..	171
3	9	4	8	..	560
4	9	4	15	..	307
Information not ascertainable					54

This revealed the fact that the largest number of infants come in the weight group of 3 lbs. 9 ozs. to 4 lbs. 8 ozs.

(e) Infant mortality :—The total number of infant deaths in institutions was 139 and that occurring at home after discharge was 13. Total deaths were 152 or 132·7 per 1,000. Of these, 90% were due to prematurity and immaturity.

Follow up.

(1) Social and economic status :—Ten of the mothers were well-to-do, 39 fairly well, and the remaining 259 were poor.

(2) Housing conditions :—8 good, 57 fair, 45 poor but clean ; housing conditions in 194 were positively bad. Over-crowding was noticed in 70% of the houses; in 3% there were as many as 21 persons to a room. The surroundings in 70% were very insanitary ; washing places, taps and water-closets were inadequate, and in certain cases situated far from the building or even absent in some cases.

(3) Income and occupation :—

(a) Income :—

TABLE V.

Salary.	Number.
Rs. 20 to 60	656
Rs. 61 to 150	144
Rs. 151 and upwards ..	48
Unemployed	42
Information not ascertainable	212

(b) Occupation :—

(i) Occupation of fathers :—The largest number viz. 468 was in service such as domestic, office peons, police, post & telegraph peons, motor and other conveyance drivers. The clerks included in this number were 115. The second-highest were millhands viz. 207. 124 petty traders such as tailors, cobblers, painters, hawkers, carpenters etc. Mechanics were 56, shopkeepers 24, large business people 28, and 1 social worker. 42 were unemployed, and in 152 cases information was not obtained.

(ii) Occupation of mothers :—Of the 1,102 mothers, only 54 were working outside their own homes. Their occupations were :

TABLE IV.

Occupation.	Number.
Millhands	20
Sweepers	8
Domestic servants ..	14
Coolies	3
Bidi shop workers ..	2
Cigarette factory workers	2
Packing distribution worker	1
Stitching worker ..	1
Teacher	1
Nurse	1
Dental Laboratory assistant	1

The remaining mothers were domestic workers and except in case of 5%, all seemed to be overworked and were up and doing from 5 a.m. to 9 p.m.

Eight mothers were unmarried, 3 widows and 2 were deserted by their husbands.

(4) Diets :—In 10% of the mothers diet was adequate and well balanced ; in other cases the average diet consumed was very poor, inadequate, and contained very little proteins, which were chiefly of vegetable origin. Fats

and mineral salts were deficient and carbohydrates in excess. Vitamins were absent. Scarcely 30% of the mothers interviewed took extra milk. The number of calories per individual averaged 1,450. The state of nutrition of the mothers was poor, that of the children in the home fair.

The collection of statistical information is in progress in spite of the difficulties under which the staff has to work in the present abnormal conditions in the city. Conveyance and transport are not regular and the staff have to spend a lot of time in obtaining information. Information not obtainable in certain cases is due to this fact. Efforts are, however, made to see that no premature and immature births in the institutions under inquiry have been left out.

FILARIASIS.

37. Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

Dr. S. Sundar Rao carried out the work up to August when he retired and the enquiry was handed over to Dr. A. K. Mukerji.

The progress of the enquiry was considerably hampered due to the disturbances in Bengal and also lack of staff, though Dr. N. V. Bhaduri devoted a considerable time to the work.

The occurrence of eosinophilic granulomata in the spleen in cases of filariasis was reported by Prof. Dhayagude of Bombay. Twenty-five specimens of spleen from filarial cases were collected, *post mortem*, from the morgue. These were sectioned and studied. In only one case was such granuloma found.

The study of the action of the antimony compounds, stibatin and anthiomaline, in cases of filariasis (*W. bancrofti* infection) was continued. Up till now these drugs do not appear to be of much value in the relief of symptoms. As regards microfilaraemia, there appears to be only a transient reduction of the number of microfilariae after their use. Observations are, however, to be carried out for a longer time and on a large number of cases before a conclusive opinion can be given.

OTHER RESEARCHES.

38. Hematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

I. Blood picture and protein content of healthy Indian vegetarians.

Subjects of study :—These were selected from among healthy adults who were strictly vegetarians from their birth. Their ages varied from 17 to 44 years. Fifty-one male and seven female subjects were examined. All were from south India and the majority belonged to the middle class.

Methods of investigation :—Complete cytological examination of blood including haematocrit reading, erythrocyte sedimentation rate, Arneth count and van den Bergh test was done in all the cases according to standard method (Haematological technique—Napier and Das Gupta, 3rd edition, 1945). In the beginning total plasma protein together with albumin-globulin ratio was estimated by the ' copper sulphate method '. Some of the results obtained with the ' copper sulphate method ' were checked against the micro-Kjeldahl method. As in some cases appreciable difference was found between the results obtained with the two methods, ' copper sulphate method ' has now been abandoned in favour of the more accurate micro-Kjeldahl method. The results are :—

	Mean.	Maximum.	Minimum.
Haemoglobin in gms. per 100 mlg. of blood ..	16·48	19·52	12·92
Red cells in millions per cu. mm. ..	5·40	6·48	4·46
Reticulocyte percentage	0·22	1·0	0·1
Mean cell volume in u^3	85·8	94·60	67·60
Mean corpuscular haemoglobin in yy ..	30·1	34·3	22·3
Mean corps. hb. concentration percentage ..	35·3	39·8	30·8
White cells per cu mm.	7439	21,900	4575
Neutrophils per cu mm.	4229	7665	2255
Lymphocytes per cu mm.	1600	4161	200
Monocytes per cu mm.	424	1155	152·5
Eosinophils per cu mm.	1206	9198	112
Basophils per cu mm.	50·08	133·0	14·5
Arneth count wt. mean	1·46	1·82	1·19
Erythrocyte sedimentation rate (Wintrobe 1 hr. reading)	8·9	32·5	1·0

A perusal of the different data shows that the blood picture closely resembles the blood picture of healthy non-vegetarian Indians except in the eosinophil counts. In 37 per cent of these individuals relative and absolute eosinophil counts were high; these are being studied further. According to micro-Kjeldahl method total plasma protein varied from 4·55 to 7·75 gm. with a mean of 6·35 gm. per cent (6·1 gm. to 8·6 gm. with a mean of 6·97 gm. per cent) ; albumin values varied from 2·83 gm. to 4·7 gm. with a mean of 3·63 gm. per cent (3·78 gm. to 5·14 gm. with a mean of 4·3 gm. per cent) ; and globulin values varied from 1·61 gm. to 3·65 gm. with a mean of 2·52 gm. per cent (1·99 to 2·9 with a mean of 2·68 gm. per cent). The figures given in brackets are the readings with the ' copper sulphate method '.

II. *Studies in eosinophilia with special reference to the pH of the blood* :—The CO_2 combining power of plasma was determined according to Van Slyke's

method in eight cases of eosinophilia (eosinophil counts varying from 781 to 5969 per cu mm.). The values varied from 39·3 ml. to 53·2 ml. with a mean value of 46·2 ml. per 100 ml. In another series of 8 cases with eosinophil counts with normal limits the values varied from 47·6 ml. to 60·6 ml. with a mean value of 54·3 ml. per 100 ml. It appears that cases with eosinophilia have lower values suggestive of slight diminution of alkali reserve of the blood. Further works in this line are in progress in collaboration with Prof. P. De, B.Sc., M.B., F.R.C.P., Professor of Physiology, Medical College, Calcutta.

III. *Work in connection with tropical macrocytic anaemia :*—Synthetic folic acid (Lacto bacillus casei factor) Lederle, reported to be highly efficacious in macrocytic anaemias was available for clinical trial. In view of the greater possibilities of this drug, it was decided to assess its role in the treatment of macrocytic anaemia, in preference to the scheme outlined in the proposal. During this period 11 cases were treated with synthetic folic acid. The results of folic acid therapy compared favourably with those obtained with liver extracts, oral (proteolysed) or parenteral. Regeneration of blood after folic acid was always quick in all the cases excepting one, and in fact appeared to be quicker than what is generally seen after liver therapy. The increased activities in the marrow was reflected in peripheral circulation at first by reticulocytosis and later by increase in the red cell count and haemoglobin values. Reticulocytosis after folic acid was a constant feature and was generally of a higher magnitude than what is generally seen after potent liver extracts, oral or parenteral.

39. Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras.

The staff of the enquiry was engaged

(I) on the evolution of a technique for the manufacture of influenza virus A vaccine and (II) in studies on the behaviour of membrane vaccine lymph in the field.

(I) *Influenza vaccine manufacture.*

Early in the year attempts were first made to propagate influenza virus A in eggs from material obtained from the U.S.A. On an experimental basis a few doses of vaccine were made. It was suggested that preliminary trials should be made at extended manufacture in order to evolve a technique for a larger scale manufacture when needed. The Government of India had agreed to meet the cost of such trials in the first instance.

At this time full details of technique of manufacture as adopted in U.S.A. were not available except certain essential details involved in the process. Precipitation of the virus by freezing and thawing and the technique of erythrocyte absorption and elution method were both studied. A suitable technique of manufacture by the latter method was worked out after studying the several factors, such as age of the embryo, suitable concentration of erythrocytes for absorption and the temperature and time factors in the absorption and elution of the virus. From further published reports it was noted that the technique evolved was more or less in agreement with that followed elsewhere.

The immunity producing properties of the vaccine were tested in mice following the technique prescribed by the South African Institute for Medical Research which was made available to us by the Public Health Commissioner. The results obtained were not, however, strictly comparable, in that the percentage mortality in control mice was not high though they had shown consolidation of lungs due to the action of the virus. Further attempts will be made in this direction after enhancing the virulence of the virus by mouse lung passages.

Diagnosis of virus influenza.

No studies have been made in India on the prevalence of types of the virus in epidemics of influenza. For this purpose the Public Health Commissioner was requested to issue a circular requesting the medical and public health authorities to send to this Institute samples of sera from suspected cases of epidemic influenza. No samples were, however, received for such tests. It is proposed to make the influenzal antigen for distribution to facilitate undertaking of such tests at suitable centres.

(II) *Vaccinia virus.*

(a) In one of the previous reports a statement was made that the reactions noted with membrane lymph were milder than those obtained with calf lymph. This aspect was studied in greater detail. 28 cases of primary vaccination in each group were studied in detail and full notes were made of day to day development in the reaction with special reference to appearance of successive stages in the development of vaccine vesicle, the size of the areola, and the final scar area. It was observed that in all cases of membrane lymph vaccination the reaction was delayed by 24 to 48 hours, at all stages till the maximum development of the pustules. At the full pustular stages the reactions with both the types of lymph were indistinguishable.

(b) Since it was decided to terminate this investigation this year, an opportunity was taken to study the immunity reactions in the membrane and calf lymph groups by cross revaccination tests, i.e., by revaccinating the initial membrane group with calf lymph and vice versa. The primary vaccination in both the groups was done four years ago. The results are as follows :— In children primarily vaccinated with membrane lymph and revaccinated with calf lymph, the immune reactions and modified reactions togther were 40·9% and frank takes 57·6%, 1·5% were failures. In other group with primary vaccination with calf lymph and revaccination with membrane lymph the corresponding percentages were 98·5 and 1·5 respectively, there being no failures. Using vaccine virus as the indicator, it would appear therefore that the resulting immunity with membrane lymph is of a shorter duration than that obtained with calf lymph. The general observation recorded in the previous year's report was thus confirmed.

(c) *Protection against smallpox.*

In the previous reports the question of protection against smallpox afforded by the two types of lymph was judged by the general incidence rate of smallpox in the groups. The occurrence of a severe epidemic of smallpox in a 'cherry' in one village provided an opportunity to study this aspect in greater detail. A house to house survey was done noting the vaccinal state of the population including the number of marks, and the incidence of smallpox in the population. The total population at risk was 1442 and there were 64 attacks and 13 deaths.

Certain broad conclusions are given here.

1. In a group of 96 children primarily vaccinated with chick membrane lymph during the last five years there was no attack of smallpox.

2. In a similar group of 118 children vaccinated with calf lymph there was one attack and no death. This case was in an adult of 25 years of age and was vaccinated 5 months previously.

3. In the unvaccinated group of 169 (all age groups) there were 19 attacks and 7 deaths.

4. The remaining cases had occurred in the population previously vaccinated with calf lymph before the commencement of the membrane lymph experiment. These data will be presented at a later date.

5. The 64 cases of smallpox had occurred in 36 houses in the area. The inmates numbered 203. This group can be considered as having been exposed to maximum intensity of infection. The analysis yielded results of interest from the point of view of vaccination in general. The relevant data from the point of view of membrane lymph show that:—

(i) In 8 cases of primary vaccination with membrane lymph there were no attacks.

(ii) In 10 cases of primary vaccination with calf lymph there was one attack. This, as stated before, was an adult of 25 years of age vaccinated 5 months previously.

(iii) In the unvaccinated group of 30 (all ages) there were 19 attacks and 7 deaths. In the age group of 0-5 years there were 8 unvaccinated children of whom 7 got the attack and 3 died.

It would appear from these data that within the period of observation of 5 years no difference was noticeable in the protection afforded against smallpox by the two types of lymph.

40. Enquiry on anaerobic wound infections under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

1. During the course of this calendar year so far ten strains of *Cl. welchii* were taken up to determine their toxic components in quantitative units. Fresh filtrate of *Cl. welchii* S.R. 12, the control strain, was obtained from its growth in selective medium. After ascertaining its M.L.D. for white mouse, varying amount of this filtrate in different dilutions were made to react with freshly prepared egg yolk solution for a fixed interval. The resultant turbidities were matched with normal grey filter density 1·5 in Leitz's colorimeter. It became possible to plot the lecithinase activity curve of this control strain. Similarly other strains of *Cl. welchii* were studied. It was noted that these strains had lost much of their original pathogenic and toxigenic capacity. It was therefore not possible to determine their toxic components quantitatively. Three strains of *Cl. welchii* were passed through animals in order to raise their virulence. These strains are in the process of study and the result obtained would be communicated later on.

2. Effect of prolonged high lecithinase content of culture medium on lecithinase production by *Cl. welchii*:

Three strains of *Cl. welchii* were grown in medium containing cooked brain pieces for a month. At the end of this interval, these strains were tested their capacity to produce lecithinase—an enzyme which is closely associated with α factor which is responsible for lethal effect. It was noted that this prolonged cultivation in this special medium did not vary their capacity to produce lecithinase.

3. Special medium for toxin production :

As "Evan's peptone" was available, a special medium recommended by MacFarlane, containing sulphated horse muscle extract, Evan's peptone, glucose in requisite quantities, was prepared. Two strains of *Cl. welchii* were repeatedly tried in this medium, for toxin production. It was found that this medium failed to exhibit enhanced effect on the toxin production of these strains.

41. Enquiry on the action of snake venoms and their constituents on the biochemical activity of tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

1. *Effect of cobra venom on the aerobic oxidation of d-glutamic acid.*

It has already been reported that snake venoms such as that of the cobra can inhibit the oxidation of glucose, lactic acid, succinic acid, pyruvic acid, etc. by the brain cells of pigeons to a considerable extent. As glutamic acid is also an important metabolite in the living cell, the effect of cobra venom on the oxidation of glutamic acid by the pigeon's brain was investigated. As glutamic dehydrogenase is specific for d-glutamic acid, the sodium salt of d-glutamic acid was used as the substrate.

The Warburg's manometric apparatus was used in these experiments. The brain, immediately after removal from the pigeon, was chopped and minced to a fine state, weighed out in portions of about 100 mgs. in the Warburg's flasks and suspended and broken up in phosphate-Ringer-Locke solution, pH 7·4. The necessary amount of cobra venom also dissolved in the phosphate-Ringer-Locke solution was added, the volume of the liquid inside the flask being 2·0 c.c. 1 c.c. of sodium d-glutamata (0·1 M) in phosphate-Ringer was kept in the side tube, so that the final concentration of the substrate in the reaction mixture was 0·033 M. 0·2 c.c. of 20 per cent KOH was put in the inner cup together with a roll of filter paper. Suitable controls were kept side by side. The flasks were fitted to the manometers, the air displaced by oxygen and the whole arrangement was shaken in a thermostat at 37·5°C. for one hour and a half in order to reduce the residual activity of the brain cells and to allow sufficient time to the venom to exert its effect on the cells. The temperature of the thermostat was kept constant within ±0·1°C. by a sensitive thermoregulator. After shaking for one hour and a half, the substrate was tipped off from the side tube in the main reaction mixture, the level of liquid in the manometers were adjusted and the rate of oxygen consumption was determined at intervals of 15 minutes for a period of one hour.

Various doses of cobra venom ranging from 0·01 mg. to 1·0 mg. were used. The results showed that 1·0 mg. of the venom produced an inhibition of 80 to 90 per cent of the oxidation of sodium d-glutamate and that the inhibition did not decrease appreciably with smaller doses of venom down to 0·1 mg. showing

that the saturating limit had been reached. With smaller doses of venom, the degree of inhibition decreased and was of the order of 40 per cent with 0·01 mg. of venom.

The experiments on the effect of cobra venom and some of its constituents on the aerobic oxidation of different substrates by the brain cells of pigeons indicate that cobra venom possesses the property of inhibiting practically all the respiratory enzyme systems of the living cell, including the cytochrome-cytochrome oxidase system, while the isolated active principles e.g., haemolysin, choline esterase, neurotoxin, etc., were without effect. An impure variety of cardiotoxin had a very strong effect, but the purified active principle showed little effect. These experiments indicated the presence of a separate factor in cobra venom which is responsible for the inhibition of the respiratory enzymes.

2. *Studies on anaerobic oxidation.*

The process of oxidation of substrates like glucose, lactic acid, succinic acid, etc., by the brain in presence of air does not involve the direct addition of oxygen to or withdrawal of hydrogen from a substrate through the specific enzyme, but the transfer of hydrogen takes place in a series of steps through the carriers like cozymase, flavoprotein, cytochrome, etc., and it is not possible to estimate the effect of cobra venom on the individual enzymes in the aerobic experiments. To eliminate some of the above-mentioned carriers and thus to get an idea of the amount of inhibition of respiratory enzymes by cobra venom, Quastel's ferricyanide technique of anaerobic oxidation was taken recourse to (Biochem. J. 1938, 32, 936).

The method depends upon the fact that in an anaerobic atmosphere in presence of ferricyanide and bicarbonate, for each equivalent of substrate oxidised, one equivalent of H+ ion is set free which drives off a mole. of CO_2 from the bicarbonate and this evolved CO_2 is estimated manometrically. The rate of evolution of CO_2 is a measure of the rate of oxidation.

Technique :—About 100 mgs. of the chopped and minced pigeon's brain were weighed in the Warburg's flasks and suspended and broken up in bicarbonate Ringer's solution, pH 7·4 ($NaCl$=0·15 M, $NaHCO_3$=0·025 M, KCl=0.006 M, $CaCl_2$=0·001 M) to which was added a solution of the adequate amount of cobra venom in bicarbonate-Ringer. 0.2 c.c. of a ferricyanide-bicarbonate solution (5 c.c. of 10% $Na_3FeC_6N_6$ solution mixed with 1·0 c.c. 0·16 M $NaHCO_3$ solution) was also added. The substrate dissolved in bicarbonate-Ringer was placed in the side tube. The flasks were filled with a mixture of 95% N_2 + 5% CO_2 and yellow phosphorus was kept in the inner cup to maintain perfect anaerobiosis. The rest of the method is the same as has been already described. In the case of oxidation of substrates other than succinate, 0·1 c.c. of 1 per cent methylene blue was used together with the ferricyanide.

The results indicate that in the case of succinic dehydrogenase, the effect of cobra venom is rather small, being of the order of 30 per cent with 0·1 mg. of venom, whilst with 0·01 mg. of venom the inhibition is nil ; the corresponding inhibitions with 0·1 mg. and 0·01 mg. of venom in the aerobic oxidation are respectively 80 to 90 per cent and 40 per cent. This indicated that the inhibition of the aerobic oxidation of succinate by cobra venom was mainly due to the action on the cytochrome-cytochrome oxidase system.

In the case of anaerobic oxidation of glucose, lactic acid and pyruvic acid, the degree of inhibition of oxidation with 0·1 mg. and 1·0 mg. of venom was

found to be about 90 per cent, the same as was found in the case of aerobic oxidation of these substrates.

3. *Comparison of the action of cardiotoxin with that of the drugs of the digitalis and saponin group.*

The isolation in the pure state from cobra venom of a specific active principle responsible for the stoppage of the movements of the heart has been already reported. As drugs of the digitalis series and the saponin group have also the property of stopping the movements of the heart, a comparative study of the action of cardiotoxin and these chemicals on isolated amphibian heart was undertaken.

It has been observed that when an excised heart is perfused with a solution containing any of the drugs such as strophanthin, digitalis, sponin or cardiotoxin, it stops and passes on into a systolic contracture. The effect of the drugs of the digitalis series and saponin group can, however, be removed by washing out with Ringer and the heart revives its normal function. As distinct from this observation, the stoppage of the heart in the case of cardiotoxin could not be restored or prevented under any circumstances by continuous washing with Ringer or Ringer containing acetyl choline or antivenin and potassium ions whose actions on isolated heart are exactly opposite to that of cardiotoxin, that is to say, the heart stops, when perfused, in diastole. This, therefore, leads us to the conclusion that the action of digitalis and saponin is reversible. It suggests also that the mode of action of cardiotoxin must be different from that of digitalis and saponin.

42. Enquiry on typhus under Dr. D. W. Soman at the Haffkine Institute, Bombay.

(I) Work on typhus in Bombay.

The investigation undertaken was chiefly concerned with the serological diagnosis of typhus fever by Weil-Felix reaction and isolation of rickettsial strains from cases whenever possible. In all sero-diagnostic tests determination of the level of normal antibodies in the local population is of primary importance, on basis of which, the antibody behaviour is studied in the presence of clinical signs. Only after consideration of such basic data (this has not been reported so far in India as far typhus is concerned), the minimum diagnostic titre for a particular disease could be established. Isolation of rickettsial strains was only attempted after the results of Weil-Felix tests were known, on the sera, usually received late in the course of disease—a procedure that made isolation extremely difficult.

(a) *Serological diagnosis.*

(i) To determine the level of normal B. proteus OX agglutinins in the local population, Weil-Felix reaction was carried out on 500 Wassermann and Kahn sera. Only 3 out of 500 sera were positive for B. proteus OX19 agglutinins in a dilution of 1 : 100 or over and none for B. proteus OX2 and OXK agglutinins.

(ii) 174 sera from typhoid fever and 11 sera from para-typhoid fever cases (bacteriologically proved by clot cultures) were studied in their relation to their

Weil-Felix titres during different stages of disease. Ony 2 sera were positive to B. proteus OX19 antigen in a dilution of 1 : 50 and 1 : 250. One sample was positive to B. proteus OX2 antigen in 1 : 50 and the other to B. proteus OXK antigen in 1 : 50. These results showed that normal Weil-Felix titres were not appreciably influenced by the course of typhoid and para-typhoid infections.

(iii) Considering the number of positive sera showing agglutination in a titre of 1 : 100 or 1 : 125 in febrile and afebrile cases, it was concluded that a titre of 1 : 125 in the early stages of typhus fever could be taken as suggestive and a titre of 1 : 250 or over as confirmatory of that condition. 1578 sera received for widal reaction, were put up as routine for Weil-Felix test. Of these 142 samples proved positive in a dilution of 1 : 250 or over to either of the three known B. proteus strains. 69 showed positive agglutination against B. proteus OX19 antigen, 8 against B. proteus OX2 and 31 against B. proteus OXK. Positive agglutination was demonstrable in 32 sera against both B. proteus OX19 and B. proteus OX2 antigens.

(b) *Isolation of strains.*

(i) In 27 cases of typhus fever, serologically confirmed, guinea-pigs were inoculated with blood, with a view to isolate the rickettsial virus. Only in 11 cases, guinea-pigs showed a febrile reaction, with scrotal swelling (Neillmooser reaction) in 2 cases only. Rickettsia were demonstrated in 4 cases. The work of confirming the diagnosis in the remaining guinea-pigs by demonstration of rickettsia or by other evidence is in progress.

(ii) A murine strain of rickettsia was recovered from Bombay rats and when studied further by cross immunity experiments using a 'murine Wilmington strain ", the local strain was proved to be of the murine type.

(iii) Attempts have been made to isolate strains of rickettsia from four cases giving Weil-Felix reaction with proteus OX2 antigen. No strains were isolated.

(iv) Studies on the maintenance of strains of rickettsia in a lyophalized state are in progress. The results obtained so far being encouraging require to be further continued.

(II) Work on scrub typhus (Mite typhus).

(a) *Serological diagnosis.*

The diagnosis was made in the first instance during the course of routine examinations of sera from pyrexial cases for evidence of typhus infection. The criteria of a positive serological diagnosis was a minimum agglutination titre of 1 : 250 against an alcoholised B. proteus OXK suspension. Out of 1578 sera thus examined, only 31 samples were found to be positive and these were derived from 17 cases, which occurred during the months of Setpember, October and November. No serologically positive sample was obtained in any other period during the year. Average maximum titre reached in these cases was 1 : 1250 except in one case where it was not above 1 : 125.

(b) *Isolation of strains.*

Rickettsial isolation was attempted in twelve cases by inoculating mice intraperitoneally with patient's blood, but only in seven cases it was successful. The mice usually became ill between 8 to 12 days and died. On post mortem

examination, a characteristic, sticky, gelatinous exudate was seen on the peritoneum; spleen was enlarged and occasionally pleural fluid was present. Smears from the peritoneal exudate showed the presence of Rickettsia orientalis in large numbers. All the strains are being maintained by serial passages in mice for future comparative study.

(c) *Clinical picture.*

All the seven cases were of continuous or remittent type of pyrexia, which terminated by lysis in 14 to 16 days. Rash was noticed in two patients. The primary eschar—the characteristic dermal lesion—was present in two cases only. Four patients were males and three were females. Their ages varied from 10 to 63, all being adults except two. Clinically five cases were mild and two of a severe type. There was only one death.

Four out of seven patients were permanent residents of Bombay city, who had, for a period of not less than one month, never been outside the city limits. Contrary to the current belief, with the isolation of rickettsia in the above four cases, the presence of scrub typhus is now definitely established in Bombay city which is a metropolitan area and this opens up a new field for investigation into the causal arthropod vector, such as the larval mite and the rodent reservoir of infection.

43. Enquiry on kala-azar under Dr. P. C. Sen Gupta at the School of Tropical Medicine, Calcutta.

(I) The complement fixation test for kala-azar.

The method of preparation of the antigen from Kedrowsky's and other acid-fast bacilli, as well as the method of titration of the antigen for the purpose of the complement fixation test according to the modified technique described by Sen Gupta have been worked out. A paper on this subject in collaboration with Dr. Dharmendra and Mr. R. Bose of the leprosy department has been published in the *I.J.M.R.*, May, 1946. Attempts are being made to prepare the antigen from the human tubercle bacillus for which purpose, the bacteriology department of the School have commenced isolation and culture of several strains of the human tubercle bacillus.

(II) Chemotherapeutic investigations on kala-azar.

1. *Therapeutic trials.*

(a) *Sodium antimony gluconate*:—Clinical trials of this drug in a series of 50 cases of kala-azar and 2 cases of post-kala-azar dermal leishmaniasis were carried out last year. The final results of this trial were analysed, after the replies to follow up letters (sent out not less than 6 months after the completion of treatment) were received or the patients re-examined after that period or earlier in case of relapses. It has been found that in the treatment of well developed cases (of duration of three months or more) this drug is as effective as the older pentavalent antimonials such as neostibosan, but the relapse rate after treatment of the early cases of kala-azar with this drug is very high.

(b) 4 : 4^1 -*diamidino diphenoxy pentane* (*Pentamidine, M & B* 800):—This compound was tried out by Napier and Sen Gupta in the treatment of

kala-azar in 1942-43, using a dosage scheme similar to that of stilbamidine. The immediate results were quite satisfactory but the relapse rate proved to be higher. A fresh clinical trial of the di B hydroxy ethane sulphonate salt of using a more intensive and prolonged course of treatment has been completed. The immediate results have been satisfactory. The final results will be ready early next year, after the follow up of the cases more than six months after treatment have been completed. This drug can be administered intramuscularly without causing much pain.

(c) Some Indian preparations of sodium antimony^{v} gluconate are at present under investigation, as well as an oily suspension of this salt, prepared by the Glaxo Laboratories.

2. *Animal experiments.*

(a) *Experiments to produce diamidino-stilbene neuropathy in the rhesus monkey :*—Three monkeys were given series of injections of diamidino stilbene (stilbamidine). Three to four months after the completion of the injections, two monkeys were destroyed by air embolism and the tissues of the central nervous system collected for histological examination. Sections from the pons of one of the animals were stained for Nissl's granulas with toluidine blue, but the results have been indefinite. Further studies are in progress.

(b) *Studies in the cumulative toxic action of diamidine stilbene in the rabbit :* This was commenced in 1943 by the officer-in-charge of this inquiry. Histological examination of the tissues of three rabbits have almost been completed. Preliminary examination carried out so far shows that extensive degenerative changes have occurred in the liver and kidney. There have been haemorrhages in the musculature of the heart, in the lung parenchyma as well as in the liver, kidney and the suprarenal. Further studies, particularly of the nervous tissues have now to be taken up.

(c) *Attempt to transmit leishmaniasis to the squirrel :*—Japanese squirrel has been reported to be susceptible to infection with leishmania. Twenty Indian squirrels were inoculated with a suspension of leishmania grown on the surface of J. C. Roy's medium. So far no evidence of leishmanial infection has been found in any of the squirrels that have died naturally or have been destroyed for the purpose of examination.

(III) Treatment of agranulocytosis complicating kala-azar.

Three cases of kala-azar who had developed agranulocytosis were treated at the Carmichael Hospital for Tropical Diseases during the period of one year. Combination of penicillin pentnucleotide, liver extract and general supportive measures and pentavalent antimonials was successful in leading to a cure of agranulocytosis in two cases. The other case which did not receive pentnucleotide, went on to a fatal termination.

44. Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

Investigation on the life-history of Gastrodiscoides hominis.

An assistant research worker was appointed on the 10th of July and given suitable training for carrying out the work in Assam. The rainy season in

Assam ends about the end of September and water from the low-lying districts begins to recede. From previous experience this part of the year appeared suitable for collection of water plants and snail fauna which are abundant and easily available and it was proposed to send the worker to Assam early in October. Owing to the disturbances in Bengal, he could not leave Calcutta earlier than 19th of November. It was found by him that the low-lying places of Barpeta had already become almost dry and the water plants scarce. A survey, however, was conducted in five villages around Barpeta and the infected persons have been located. Eggs of *G. hominis* have been concentrated from the stools of these persons and left to hatch. Hatching has not taken place in three weeks and collection of eggs are being continued. Available local snails, mostly hibernating now and few in number, have also been collected and exposed to the eggs and are under observation for the possible development of cercaria.

Indigenous anthelmintic drugs.

This work was concluded this year. The drugs tested were *Butea frondosa, Embelia ribes* and *Kamala*. The drugs were finely powdered and given singly or in combination with one another. The result of treatment is shown below. The numbers against the drugs show the number of persons treated and those in brackets show the number cured.

Hookworm infestation:—Butea, 15-120 grs. 32 (1), Embelia, 60-180 grs. 11 (1), combined Bueta and Embelia, 15-180 grs. of each, 153 (13). The value of these two drugs is, therefore, negligible against hookworms.

Ascaris infestation:—Butea, 8-120 grs., 36 (12), Embelia, 30-180 grs., 12 (4), combined Butea and Embelia, 10-180 grs. of each, 232 (71), combined Butea, Embelia and Kamala, 10-60 grs. of each, 25 (5). Analysing the figures it was seen that the cure rate was better with the doses of 60 grs. and over than with those below 60 grs. Butea, 60-120 grs. cured 8 of 12 cases, Embelia, 60-180 grs. cured 4 out of 10 cases, combined Butea and Embelia cured 47 out of 97 cases and combined Bueta, Embelia and Kamala cured 3 out of 6 cases. About half the numbr of cases were cured by one treatment with 60 grs. or more of these drugs. This result compares very favourably with that of santonin and oil of chenopodium. In a series of 90 cases, one dose of santonin grs. 3, cured 31 cases only and in another series of 67 cases, 37 were cured by one treatment with 1·5 c.c. of oil of chenopodium. Butea and Embelia are, therefore, better than santonin and are equally effective as oil of chenopodium against Ascaris. Working out the relative cost of these drugs, it was found that a dose of two drachms of either Bueta or Embelia costs less than one pice while one 3-grain dose of santonin costs three annas and one 1·5 c.c. dose of oil of chenopodium about two annas. Butea and Embelia are, therefore, effective and cheap substitutes for the above imported drugs.

Taenia (tapeworm) infestation:—Embelia, 60-180 grs., 17 (3), combined Butea and Embelia, 90-180 grs. of each, 4 (0), combined Embelia and Kamala, 30-120 grs. of each, 13 (0). These drugs have little effect against tapeworms. Sixteen cases of *Hymenolepis nana* infestation were also treated with these drugs singly or in different combinations with no cures.

45. Enquiry into methods for the manufacture of penicillin and other problems connected with it under the Director, Haffkine Institute, Bombay.

Growth of *penicillium notatum*.

A number of media, 24 in all, were tried to ascertain the highest penicillin yields and the best conditions for the growth of the mycelium Penicillium notatum using two strains, viz. "N.R.R.L." and W. & B., the depth of the medium being 1·5-2·0 cm. for maximum production. The media were sterilized by autoclaving at 15 lb. pressure for 20 minutes and then inoculated with spore suspensions in sterile distilled water from cultures on Czapek-Dox agar and incubated at 24°C. for 12-14 days, after which they were extracted.

The initial pH of the media were between 6.0-7.0, but came down to 3-4 by the third day. By the 4th or the 5th day the pH then rose to 5·0. From the 6th day onwards, the pH began to rise with the colour change in the medium which was colourless in the beginning, turned light yellow on the 5th day and subsequently golden yellow with the rise of pH with the maxium of 8·8; but penicillin production was maximum at about pH 7·0 by the 12th day.

It was noticed that growth after 14 days became dry, powdery and dull greyish in colour indicating that sporulation had commenced.

Whereas the W. & B. strain gave the typical bluish green growth within a week, the N.R.R.L. was slow growing and did not form a complete mat, the growth starting from the circumference.

Although the growth was visible on each of the media tried, it was observed that the addition of corn steep liquor, bran and brown sugar enhanced the growth of the mould and thus increased its yield of penicillin.

Comparison of the relative merits of the three methods of production of penicillin from the culture fluid.

Three different extraction methods were tried and knowledge was gained as to which method was simpler and at the same time yielded a higher quantity of the antibiotic.

For the sake of comparison a few batches were extracted by all the three different methods. The following techniques were used.

(a) *Clayton, Hems, Robinson, Andrews & Hunwicke's Isolation Method.* (Ref. Biochem. J., Vol. **38**, p. 452), 1944.

After 14 days' incubation, the culture fluid is filtered through muslin and centrifuged. 5 gms. of Norit A charcoal is added to each litre of the metabolism solution and filtered. After filtration, the charcoal is washed with a little water, followed by chloroform. The penicillin is eluted by running through the charcoal a volume of 20% acetone equal to 1/10th of that of the original metabolism solution. The elute is acidified to pH 2·5 to 3·0 with 10% phosphoric acid and emulsified by rapid stirring with an equal volume of chloroform. The chloroform layer is separately collected and shaken with such an amount of 0·5% $NaHCO_3$ (Sodium bicarbonate) till the pH of the aqueous phase is 7·0. The above aqueous solution of sodium penicillin is collected in a sterile flask and kept in the refrigerator.

(b) *Berger's Isolation Method* (Ref:— Am. J. of Clin. Pathology, Vol. 15, p. 339, 1945).

After 14 days' incubation, the culture fluid is filtered through muslin and centrifuged. The pH of the clear culture fluid is adjusted to 6·4 with 20% H_3PO_4 and kept in the ice chest. 400 gms. of ammonium sulphate is added to each litre of the original metabolism solution. After 5 to 10 minutes when the Ammonium Sulphate is dissolved the voluminous precipitate which forms is filtered off through a quick filter paper. To five volumes of the culture fluid filtrate is added one volume of butanol. The mixture is shaken in a separating funnel and allowed to stand for a few minutes. The lower layer which consists of the extracted culture fluid is then drawn off and discarded. The pooled butanol extract dried by anhydrous sodium sulphate and the dry liquid passed over aluminium oxide in a Chromatographic Tube. The penicillin is eluted from the aluminium oxide by water and the eluate is acidified to pH 2·5 to 3·0 with 10% Phosphoric Acid. This acidified elute is emulsified by rapid stirring with an equal volume of chloroform. The chloroform layer is separately collected and shaken with such an amount of 0·5% sodium bicarbonate till the pH of the aqueous phase is 7·0. The above aqueous solution of sodium penicillin is collected in a sterile flask and kept in the refrigerator.

(c) *Best's Isolation Method* (Ref. Personal communication from Prof C. H. Best).

After 14 days' incubation, the culture fluid is filtered through muslin and centrifuged and kept in the ice chest. The pH of the culture fluid is adjusted to 2·0 to 2·5 with 10% phosphoric acid or sulphuric acid. It is then extracted with 0·4 to 0·5 volume of amyl or butyl acetate (92%). After allowing 2½ to 5 minutes contact time, it is centrifuged and the acetate layer containing the penicillin is collected. Penicillin from this acetate layer is transferred to the aqueous phase by using phosphate buffer (pH 7·0) or sodium bicarbonate (up to 0·4 N) by bringing the pH to 7·0. The aqueous layer is then transferred to chloroform by acidifying to pH 2·0 to 2·5 (1 : 1 aqueous to solvent ratio). The chloroform layer is separated and treated with 1% charcoal Norit A for 5 to 10 minutes when the impurities are adsorbed. (Longer contact and longer percentages will adsorb penicillin, so it should be avoided). After the contact time, the liquid is filtered off and the chloroform solution separately collected and shaken with such an amount of 0·5% sodium bicarbonate till the pH of the aqueous phase is 7·0. The above aqueous solution of sodium penicillin is collected in a sterile flask and kept in the refrigerator.

In contrast to the other methods, extraction of penicillin from the culture fluid by butanol as in method (b), outlined above, can be carried out at a pH at which penicillin is stable. Prior to extraction a suitable quantity of ammonium sulphate is added to the culture fluid. This is carried to (i) precipitate inactive pigments by ammonium sulphate, (ii) ensure more complete extraction of penicillin by butanol in the presence of ammonium sulphate and (iii) save considerable quantities of butanol which is insoluble in aqueous solutions containing ammonium sulphate.

Penicillin contained in the butanol extract is extracted with chromatographic absorption on to a column of Brockmann's alumina. By the application of this method of extraction and re-extraction in conjunction with chromatographic absorption on alumina a still more highly purified and concentrated solution of penicillin in the form of its sodium salt was obtained. This solution

was reddish orange in colour, becoming light yellow on dilution, and having a faint characteristic smell and bitter taste.

To test the potency of penicillin obtained, assays were carried out by the serial dilution method (Schmidt and Andrew Moyer, J. Bact., Vol. **74**, No. 2, 1944) and the Oxford Cup Method (N. J. Heatley, Biochem. J., Vol. **38**, No. 1, 1944) using S. aureus 209-P as the standard test organism received from National Type Culture Collection.

REPORTS OF ADVISORY COMMITTEES.

REPORT OF THE CHOLERA ADVISORY COMMITTEE, JULY, 1946.

The Committee met at 10-00 A.M. on Sunday, the 21st July, 1946 at the Imperial Secretariat, New Delhi.

PRESENT.

Members : Lieut.-Colonel C. L. Pasricha, I.M.S. (*Chairman*).
Dr. B. B. Dikshit.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Rao Bahadur Dr. K. C. K. E. Raja.
Lieut.-Colonel M. L. Ahuja, I.M.S., (*Secretary*).

Invitees : Major T. D. Ahmad, I.M.S.
Lieut.-Colonel A. N. Chopra, I.M.S.
Major J. R. Dogra, I.M.S.
Major A. N. Duggal, I.M.S.
Dr. A. N. Goyle.
Major M. Jafar, I.M.S.
Dr. D. L. Shrivastava.
Lieut.-Colonel Jaswant Singh, I.M.S.
Dr. P. M. Wagle.

The Committee considered the work carried out by the following enquiries under the Indian Research Fund Association :—

(a) *Cholera treatment enquiry under the Director, School of Tropical Medicine, Calcutta.*

Lieut.-Colonel Pasricha pointed out that, owing to the absence of any serious outbreak of cholera in Bengal since the beginning of this year, the number of cases treated with sulphaguanidine and sulphadiazine were few and no definite conclusions could be drawn, on the figures available, regarding the therapeutic value of sulpha drugs in the treatment of cholera.

(b) *Cholera treatment enquiry under the Director, King Institute, Guindy.*

Dr. C. G. Pandit explained that, owing to the absence of cholera in Madras, no work on the therapeutic value of sulpha drugs could be undertaken. Basic research on the growth requirements of *V. cholerae* in synthetic media was in progress and the results were encouraging. Attempts were also being made to establish infection in experimental animals. The Committee stresed the desirability of continuing basic research of this nature.

The Committee recommended that the Director, School of Tropical Medicine, Calcutta, should cooperate with the Directors of Public Health in Bihar and Orissa and send field units for trials of sulpha drugs whenever an outbreak of cholera was anticipated in those areas.

(c) *Preparation of monospecific high titre cholera sera under the Director, King Institute, Guindy.*

Dr. C. G. Pandit reported that adequate quantities of monospecific high titre cholera sera were now available for issue. He undertook to supply the sera for research purposes to cholera workers.

(d) *Enquiry on cholera under the Director, Central Research Institute, Kasauli.*

Dr. D. L. Shrivastava explained that attempts were being made to isolate the glucolipid and polysaccharide fractions of cholera with a view to study the immunological importance of these substances.

The Committee heard with interest Dr. Dikshit's account of the test employed hy him for the study of cholera toxin.

The Committee was informed that the report of Dr. Chandrasekaran on testing the validity of data collected by the I.R.F.A. in Madras Presidency on the statistical evaluation of anti-cholera inoculation, will be available shortly.

2. The Committee considered the proposal by the Government of Assam that an enquiry should be undertaken by the I.R.F.A. to map out the foci of endemicity of cholera in the Province. The Director of Public Health, Assam, informed the Committee that he had collected all relevant data regarding the incidence of cholera in the various districts of Assam but he would like the assistance of the I.R.F.A. for the statistical study of these data. A discussion followed and Dr. Raja pointed out that he had been interested, for some time past, in the problem of cholera endemicity and that he had been associated with statistical investigations into that problem at the All-India Institute of Hygiene and Public Health, Calcutta, during the period 1934-37 and later in 1942 and 1943 while working in the Public Health Commissioner's Office in New Delhi. The method of approach suggested by the Calcutta enquiry was on an elaborate basis and, when he took up the same studies at New Delhi a few years later, he felt that the process of investigating cholera endemicity could be simplified. In the period intervening between the studies at Calcutta and at New Delhi a comprehensive review of the results of the cholera studies, bacteriological and epidemiological, undertaken under the auspices of the Indian Research Fund Association during 1934-40 was published by Major-General Sir John Taylor. This review made it clear that, on the available evidence, the mode of transmission of cholera should be ascribed to case to case transfer of infection and not to its persistence, for prolonged periods, either in human beings or in the environment. If this view be accepted it seems reasonable to assume that endemicity in the true sense of the term, namely, the uninterrupted persistence of infection in specific localities, is unlikely to be a characteristic of cholera. From the administrative point of view the importance of investigating cholera endemicity lies in the desirability of separating areas, in which the disease occurs frequently, from those where its appearance takes place at larger intervals, the purpose in view being that of concentrating preventive measures, including an improvement of the environment, in the former type of areas. Towards this end relatively simple methods of comparing cholera incidence in different localities should suffice and, in the 1943 report of the Scientific Advisory Board of the Association, Dr. Raja put forward such a method on pages 7-9 of the report. It is sufficiently simple to be applied with ease by any public health worker without special training in statistics. If, on the other hand, the Cholera Advisory Committee considered that the problem of cholera endemicity should be investigated still further in its theoretical aspects, an obvious choice for a

suitable place for such investigation would be the Statistical Department of the All-India Institute of Hygiene and Public Health, Calcutta under Professor Chandrasekaran.

In the light of this discussion the Director of Public Health, Assam, withdrew his request for help from the Association and agreed that he would try to carry out the investigation independently with such help, if found necessary, as could be obtained from Professor Chandrasekaran of the All-India Institute of Hygiene and Public Health, Calcutta.

The Committee noted that samples of the Haffkine Institute cholera vaccine had recently been received by the Directors of the Central Research Institute, Kasauli, King Institute, Guindy and Pasteur Institute, Shillong. The Committee recommended that trials of this vaccine should be undertaken at an early date.

REPORT OF THE CHOLERA ADVISORY COMMITTEE, DECEMBER, 1946.

The Committee met at 2-30 P.M. on Wednesday, December 11th, 1946, at the Haffkine Institute, Bombay.

PRESENT.

Members : Lieut.-Colonel H. W. Mulligan. (*Chairman*).
Dr. B. B. Dikshit.
Dr. C. G. Pandit.
Lieut.-Colonel C. L. Pasricha.
Lieut.-Colonel Sir Sahib Singh Sokhey.
Rao Bahadur Dr. K. C. K. E. Raja.
Dr. D. L. Shrivastava.
Dr. K. V. Venkatraman. } (*Joint Secretaries*).
Lieut.-Colonel M. L. Ahuja. }

The following also attended.

Rao Bahadur Dr. R. Adiseshan.
Major T. D. Ahmad.
Professor C. Chandrasekar.
Lieut.-Colonel A. N. Chopra.
Colonel Sir Ram Nath Chopra.
Dr. P. V. Gharpure.
Dr. M. K. Habu.
Major M. Jaffar.
Major B. N. Khan.
Dr. V. R. Khanolkar.
Professor K. V. Krishnan.
Dr. M. N. Lahiri.
Mr. V. K. Parthasarathy.
Major T. C. Puri.
Dr. D. W. Soman.
Major B. L. Taneja.
Dr. P. M. Wagle.
Mr. P. Bruce White.

1. The Committee considered the work carried out by the following enquiries of the Indian Research Fund Association :—

(a) *Cholera treatment unit under the Director, School of Tropical Medicine, Calcutta.*

Colonel Pasricha explained that on account of the very low prevalence of cholera in the province during the year no extensive field trials could be undertaken. In two series of 571 and 304 cases respectively treated in the Calcutta hospitals in which the comparative value of sulfaguanidine, sulfasuxidine and sulfadiazine was investigated, it was noted that many of the cases included in the series ' were undoubtedly intestinal fluxes due to other causes than cholera ' and only about 30·1% cases were bacteriologically positive. The mortality rates in the controls were 3·7% and 0·97% respectively. The question was raised

whether in view of the very low case mortality rates prevailing in Calcutta, it would be desirable to continue the work there. Colonel Pasricha said that the unit was ready to move at short notice to a more favourable location in the event of there being an outbreak of cholera, but no opportunity had arisen during the current year. After discussion it was recommended that the enquiry based on the School of Tropical Medicine, Calcutta, should be continued. The possibilities of working in areas where a more severe type of the disease was prevalent should be explored and, in the absence of suitable clinical material, the personnel of the enquiry should devote their time to one or other of the research problems recommended for study at the Committee meeting held in Simla during June, 1945.

(b) *Cholera treatment unit under the Director, King Institute, Guindy, Madras.*

Dr. C. G. Pandit explained that on account of the complete absence of cholera in Madras during the year no field trial or trials under hopsital conditions could be attempted. Attempts were, however, made to establish intestinal infections with *V. cholerae* in laboratory animals. The method of approach was to cause initial damage to the intestinal mucous membrane of monkeys by injections of hot water or by X-irradiation of the intestinal mucous membrane and subsequently to administer a heavy dose of cholera vibrios by intra-intestinal injection. While the results were not conclusive, it was found that in 3 of 11 monkeys utilised a transient diarrhoea was set up and *V. cholerae* could be recovered from the stools on the third day after injection.

The Committee recommended the continuation of the enquiry.

(c) *Supply of monospecific high titre sera from the King Institute, Guindy, Madras.*

Dr. K. V. Venkatraman pointed out that this so called "enquiry" was in reality a service. It was now possible to comply with reasonable demands for high titre monospecific sera. Some difficulty had been experienced in obtaining Inaba sera of high titre owing to loss of titre during absorption; more work on this point was required and was in progress.

The Committee recommended the continuance of the enquiry.

(d) *Enquiry on cholera at the Central Research Institute, Kasauli.*

Dr. D. L. Shrivastava gave a brief account of the preliminary work done by this enquiry and outlined the proposed programme of work for the ensuing year. Polysaccharide complexes have been prepared from both Inaba and Ogawa subtypes of *V. cholerae.* The occurrence of the glucolipid complexes are being studied from the point of view of the occurrence in vibrios isolated at various stages of epidemic cholera, and their antigenic properties are being investigated. Mr. Bruce White who is currently collaborating in these studies supported the enquiry which, he said, was being conducted on a sound basis.

The Committee recommended the continuance of this enquiry.

2. Dr. B. B. Dikshit gave an interesting account of experiments which he had conducted at the Haffkine Institute, Bombay. A summary of the experiments is given in *Appendix* I to this report.

The Committee expressed the hope that Dr. Dikshit should continue and expand these researches but Dr. Dikshit explained that for the present, he was not in a position to do so. He hoped, however, that he would be able to resume work on similar lines at a later date.

3. *Reports of trials of cholera vaccine prepared at the Haffkine Institute :-*

The Committee recorded a report by Lieut.-Colonel Ahuja on the subject. Dr. C. G. Pandit pointed out that he would carry out comparative trials of this vaccine and agar grown vaccine, on the line adopted by Colonel Sokhey as soon as a sufficient number of mice were available.

4. The Committee considered a report on the evaluation of anti-cholera inoculation as a personal prophylactic against cholera and its efficacy in the prevention and control of cholera epidemics by Dr. R. Adiseshan, lately Director of Public Health, Madras, and a supplementary report on the statistical analysis of the data relating to 63 *cheris* in South Arcot District by Dr. C. Chandrasekar, Professor of Statistics, All-India Institute of Hygiene and Public Health, Calcutta.

The Chairman explained that Dr. Adiseshan's original report on this subject had been presented two years ago but, at that time, certain doubts had been expressed regarding the reliability of the type of basic data on which the report was based and the validity of the conclusions drawn. It had, therefore, been decided, before adopting the report, to initiate an enquiry into the reliability of these basic data by an independent worker. Dr. Chandrasekar proceeded to Madras under the auspices of the Indian Research Fund Association for this purpose and, in due course, reported that the basic data on which Dr. Adiseshan's report was based had been carefully recorded and were substantially accurate and reliable. Since then these data had been subjected to the closest scrutiny and it had now been established beyond doubt that, from the point of view of the conclusions drawn in Dr. Adiseshan's report, any omissions and inaccuracies which had been detected were of a minor nature and must be regarded as inconsequential. At the same time Dr. Chandrasekar had undertaken a statistical analysis of the data relating to a limited population of 63 *cheris* in the South Arcot District of the Madras Presidency, using a smaller and more homogenous unit, the *cheri*—than the village or hamlet used by Dr. Adiseshan. Although the results of Dr. Chandrasekar's analysis indicated a lower degree of protection than that reported by Dr. Adiseshan, they confirmed Dr. Adiseshan's main conclusion that anti-cholera inoculation affords a significant degree of protection against cholera.

Having heard the testimony of members of the Committee and others who had been intimately associated with the close scrutiny of the basic data on which Dr. Adiseshan's report was based, the Committee unanimously adopted Dr. Adiseshan's report and the supplementary report by Dr. Chandrasekar. It was agreed that these reports should now be prepared for publication in the Indian Journal of Medical Research and the authors undertook to do so at an early date. The Committee congratulated Dr. Adiseshan and Dr. Chandrasekar on the work which they had done and expressed their thanks to Dr. Raja and others who had spent much time and labour in scrutinizing the basic data.

5. Dr. R. W. Linton explained that particulars regarding the minimum requirements of cholera vaccine and the method of assay used in the United States had been supplied by him for the information of the Committee. Since then, however, the method of assay had become outmoded by the method introduced by Lieut.-Colonel Sir Sahib Singh Sokhey in India. Colonel Sokhey stated the details of his method of assaying cholera vaccine would be published shortly but that, in the meantime, he would make the latest information available to workers in India. The Committee agreed that Colonel Sokhey's method

of assay should be adopted as the method of choice for comparative tests on the value of cholera vaccine.

6. The Committee considered Section 3(a) (1) (a) and (b) of the minutes of the fifth meeting of the Medical Research Liaison Committee for India held in London on 10th September, 1946. Regarding the suggestion about the use of A type phages, Mr. Bruce White, who was present at the meeting said that he hoped while in India to investigate the possibility of using A type bacteriophage to differentiate Ogawa and Inaba subtypes of *V. cholerae*. He would be glad to receive A type phages from workers in India.

7. The Committee extended a welcome to Mr. Bruce White who was visiting India at the invitation of the I.R.F.A.

APPENDIX I.

Summary of work done at the Haffkine Institute Bombay by Dr. B. B. Dikshit.

The Cholera Advisory Committee at its meeting held in June 1945 at Simla gave a general recommendation to investigate on the problem of experimental production of cholera in laboratory animals and also to do work on chémotherapy of cholera and cholera toxin. The following is the summary of work done in the Haffkine Institute from June 1945 to June 1946. The work was done in collaboration with Dr. C. G. Pandit and Dr. V. R. Khanolkar.

(A) *Cholera in experimental animals.*

These investigations were based on the teaching of the late Professor A. J. Clark of Edinburgh who thought intestinal antiseptics so damaged the intestinal mucosa that it lost to a considerable extent its natural power of defence against invading micro organisms. Damage to the intestinal mucosa by chemical agents was obviously not practicable for the present investigation. Physical agents were therefore tried and hot water at 56° C. was used. About 10 c.c. of hot tap water at 56° C. was introduced directly into the intestinal lumen at a point approximately midway between the pyloric and iliac sphincters in rabbits. A suspension of cholera vibrios was then introduced into the intestines after an interval varying between ½ to 24 hours. These experiments gave irregular results but in two experimens out of 15 the animals developed profuse diarrhoea and died. Cholera vibrios were present in large number in the stools. Dr. C. G. Pandit who was shown these experiments thought that selection of a proper strain may influence the results and undertook to do the experiments in Guindy. Dr. V. R. Khanolkar suggested that intestinal mucosa could also be damaged by X-ray irradiation and monkeys should be used for such experiments. Dr. Pandit undertook to do these experiments also in Guindy.

(B) *Chemotherapy of cholera.*

The following points were thought to be important in preparing new chemotherapeutic drugs for cholera:—

(i) The drug must be soluble to a certain extent at a pH of about 7·8.

(ii) It should be absorbed to a very limited extent from the intestinal tract.

(iii) It should attain a concentration in the intestinaes which should be more than that required for inhibition of cholera vibrios.

(iv) It should preferably have a selective action on the vibrios.

(v) It should be possible to maintain a uniformly high concentration in the intestines by adequate spacing of doses.

With the help of Dr. H. S. Mahal of the Pharmacology department of the Haffkine Institute a chemical compound which is a combination of sulphanilamide and formaline was prepared which satisfied the first three conditions. This drug is for the time being designated as 'FSA PPt'. In the test tube it shows a bacteriostatic action on cholera vibrios which is more marked than that of sulphaguanidine or sulphasuxidine. It is absorbed to a certain extent but there is reason to believe that the concentration in the blood will not exceed about

4 to 5 mg. per cent. The concentration attained in the faeces is more than 5 times required for inhibition of vibrios. There does not appear to be any selective action on cholera vibrios and the exact dosage and spacing of doses will have to be worked out in cholera cases. The following dosage is recommended to start with. First dose 4 grammes, subsequent dose 2 grammes every 4 hours. A sufficient quantity of the drug enough to treat about 40 cases has already been forwarded to the Guindy Institute.

(C) *Cholera toxin.*

(i) Toxicity of cholera stools :—Filtered cholera stools do not kill white mice (25-28 gms.) when quantities up to 2·0 c.c. are injected intraperitoneally.

(ii) Effect on capillary permeability :—

(a) Filtered cholera stools do not increase the permeability of capillaries of rabbits skin as tested by the Trypan blue method.

(b) Living vibrios increase the permeability but vibrios killed of 55°C. for 30 minutes do not.

(c) At Dr. Pandit's suggestion some work was carried out to see if the virulence of vibrios could be determined by studying their power of increasing the capillary permeability. No conclusion could be drawn from the few experiments made.

(d) The effect of drugs on the increased permeability of capillaries caused by living Cholera vibrios was studied. It was found that FSA ppt modified the action of vibrios more than sulphaguanidine, sulphasuxidine.

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 2-30 p.m. on July 20, 1946. It was constituted as:

Major-General Sir Gordon Covell. (*Chairman*).
Rai Bahadur Dr. A. C. Banerjea.
Dr. D. K. Viswanathan.
Lt.-Col. Jaswant Singh. (*Secretary*).

The following were also present:—

Major T. D. Ahmad.
Lt.-Col. J. H. Cater.
Lt.-Col. A. N. Chopra.
Dr. S. Dakshinamurty.
Major A. N. Duggal.
Dr. A. N. Goyle.
Major M. Jafar.
Dr. I. M. Puri.
Major T. C. Puri.
Major R. Senior White.
Lt.-Col. G. Verghese.

PROCEEDINGS.

I. While congratulating Major-General Sir Gordon Covell on the conferment of Knighthood; Lt.-Col. M. K. Afrdi on the conferment of C.B.E.; Lt.-Col. Jaswant Singh on the conferment of O.B.E. and Dr. I. M. Puri on the conferment of M.B.E., the Committee noted with great pleasure the appreciation of the work done at the Malaria Institute of India.

II. The Committee expressed sorrow on hearing of the sad news of the death of Rai Bahadur Dr. B. M. Das Gupta.

III. Major-General Sir Gordon Covell gave a short resume of the work carried out on paludrine and other synthetic drugs and narrated his impressions in this connection of his recent tour in the U.K.

The drugs reviewed included besides mepacrine and pamaquine the sulpha drugs, santochin (6911), resochin (7618), SN 13276, M 4430 and M 4888 (paludrine). The sulpha drugs though possessing some plasmodicidal value, have a number of defects which make them unsuitable for general use in the treatment of malaria. Both santochin and resochin belong to the 4-aminoquiniline series. They have an action similar to that of mepacrine but are more effective and do not give rise to discoloration of the skin. SN 13276 belongs to the 8-aminoquiniline series. Its action is similar to that of pamaquin but is too toxic for general use in India. M 4888 has a totally different composition from the other drugs. It is a double guanidine and is superior to M 4430 which has now practically been superseded.

He then referred to the very important work undertaken by Brigadier Hamilton Fairley at Cairns. Comparative tests on paludrine and mepacrine indicated that paludrine was in every respect superior to mepacrine and as the result of his investigations the Australian forces have ceased to use mepacrine. He found that daily dose of 100 mgm continued for 3 weeks after exposure to heavy infection with sporozoites of *P. falciparum* through the bite of mosquitoes resulted in complete freedom from any symptoms of malaria. Parasites were not seen at any stage and sub-inoculation of 200 c.c. of blood from the 7th day

onward failed to produce infection among the recipients, a property possessed by no other drug hitherto tested. After the three weeks' treatment, parasites did not appear in circulation indicating that true causal prophylactic action is complete involving the destruction of pre-erythrocytic phase of the parasites. If paludrine is given 3 hours before an infective bite it shows no causal prophylactic action showing that the drug has little if any action on the sporozoites. A single dose of 50-100 mgm from 48 to 120 hours after exposure to an infective bite acts as a true causal prophylactic, 1 tablet of 100 mgm twice weekly completely preventing the incidence of malignant malaria.

In *P. vivax* infections, one tablet of 100 mgm twice a day will result in freedom from symptoms during treatment but 24 to 33 days after stoppage of treatment overt malaria does occur. Sub-inoculation from 9th day onward failed to infect recipients showing that there is inhibitory action against the pre-erythrocytic phase. Paludrine is therefore only a partial causal prophylactic against this parasite.

Schizonticidal action :—100 mgm paludrine thrice daily for 10 days will make *P. falciparum* completely disappear from circulation resulting in radical cure (104 out of 105 cases treated).

For the treatment of B.T. malaria dosage of 300 mgm daily for 10 days followed by 1 tablet of 100 mgm once a week for 6 months is recommended. A certain number of radical cures have been reported but relapses occur.

Action on gametocytes :—This drug does not prevent gametocytes production but gametocytes from a patient receiving paludrine do not undergo growth in the mosquito beyond the oöcyst stage and the mosquito is therefore rendered non-infective.

Toxicity :—The difference between the effective therapeutic dose and the toxic dose is very considerable. One gramme of paludrine a day may cause irritation of the gastro-intestinal and genito-urinary tracts. Symptoms rapidly disappear on reduction of dosage or subsidence of the overt attack of malaria. The maximum therapeutic dose usually recommended is only 0·3 gramme a day.

IV. The Committee discussed the following papers :—

(1) Section 6 of the minutes of the second meeting of the Medical Research Liaison Committee for India held at the India Office on 16-1-46.

(2) Preliminary report on the therapeutic trials with M4888 by Dr. A. R. D. Adams and Prof. B. G. Maegraith issued by the Therapy Sub-Committee of the Malaria Committee of the M.R.C.

(3) Minutes of the meeting of the Therapy Sub-Committee of the Medical Research Council Malaria Committee held on 15-12-45.

(4) Minutes of the meeting of the Therapy Sub-Committee of the Medical Research Council Malaria Committee held on 20-3-46.

(5) Paper on " The estimation of paludrine in blood " by Prof. E. J. King and his colleagues.

The Chairman referred to the experiments on paludrine proposed to be carried out in India at different centres emphasizing the need for strict supervision ensuring consumption of the drug and maintaining records of cases with their follow-up. The Directors of Public Health of the provinces expressed their willingness to co-operate in carrying out these trials. The Chairman assured them full assistance in this respect.

REPORT ON THE MALARIA ADVISORY COMMITTEE.

The Committee met at 10-00 a.m. on December 11, 1946, at the Haffkine Institute, Parel, Bombay. It was constituted as :—

Major-General Sir Gordon Covell. (*Chairman*).
Lt.-Col. M. K. Afridi.
Dr. B. Ananthaswamy Rao.
Rai Bahadur Dr. A. C. Banerjea.
Bt.-Col. Sir Ram Nath Chopra.
Dr. M. O. T. Iyengar.
Lt.-Col. H. W. Mulligan.
Dr. D. K. Viswanathan.
Lt.-Col. Jaswant Singh. (*Secretary*).

Lieut.-General R. Hay, Chairman of the Scientific Advisory Board of the Indian Research Fund Association very kindly attended the meeting.

The following were also present :—

Major T. D. Ahmad.
Lt.-Col. A. N. Chopra.
Dr. P. V. Gharpure.
Dr. A. N. Goyle.
Dr. B. C. Das Gupta.
Major M. Jafar.
Dr. M. M. Khan.
Lt.-Col. C. K. Lakshmanan.
Dr. M. S. Muktha Sen.
Major N. L. Mukherji.
Lt.-Col. C. L. Pasricha.
Dr. I. M. Puri.
Major T. C. Puri.
Prof. K. Subrahmanyan.
Major O. P. Verma.

Proceedings.

1. The reports on enquiries carried out during the current year and proposals for 1947-48 were considered.

I. Existing enquiries.

1. *Insecticide and Mosquito Repellent Enquiry under the Director, Malaria Institute of India.*

Work on the preparation of DDT emulsion concentrate has been continued. The object has been to produce a concentrated solution miscible with water in very high dilutions using medium kerosene extract (MKE) and/or turpentine as solvents. Various emulsifiers have been tried and an emulsion concentrate containing 35 per cent DDT has been prepared with MKE as a

solvent. This, unlike the turpentine-toluene emulsion concentrate, is not highly inflammable. The different emulsions prepared have been subjected to various laboratory and field tests.

As preliminary experiments had shown that DDT suspension in water, when applied as a residual spray against adult mosquitoes, is as effective as DDT solutions or emulsions, efforts were concentrated on the preparation of a suitable suspension. A method has been evolved, but the highest concentration of DDT so far obtained in the stock suspension does not exceed 50 per cent. Work on these lines is being continued with the object of developing a stock suspension of greater concentration.

For the application of indoor residual spray, a stirrup pump fitted with a lance provided with a wheel-cock at one end and a fine nozzle with an orifice 1/32-1/64 inch in diameter at the other, has been found more satisfactory than any other type of spraying equipment. The output of such a nozzle is 8 to 12 oz. per minute, and it takes one unit (two men operating one stirrup pump) about 12 minutes to spray a room of 1,000 square feet wall surface. The inclusion of a simple pressure relief valve designed at the Malaria Institute of India as an integral part of the main body of the apparatus has greatly increased its efficiency.

A number of experiments have been carried out in the laboratory and in the field to test the comparative efficacy of different doses of DDT solution, emulsions and suspensions and of gammexane solutions as residual sprays.

Field trials were carried out in certain villages in Delhi Province and in Baluchistan. From these it was concluded that:—

(i) Indoor residual spraying is the most effective method. Outdoor residual spraying is not effective either against *A. culicifacies* at Delhi or against *A. superpictus* at Quetta.

(ii) For residual spraying, emulsions (oil in water) and suspensions (in water) containing 1·25 per cent DDT and applied at the rate of 2 c.c. per square foot are highly effective. One application in the Quetta (*superpictus*) area and two or possibly three, in the Delhi (*culicifacies*) area are probably sufficient to control the incidence of malaria during the whole malaria season.

Proposals for 1947–48.

(a) Systematic testing of insecticidal sprays, repellents, larvicides, under field conditions against various vector species of mosquito in different parts of India.

(b) Testing of spraying apparatus under different field conditions.

(c) Observations on the bionomics of the vector species of mosquito in different parts of India.

2. *Mammalian Malaria Enquiry under the Director, Central Research Institute, Kasauli.*

(i) *Mosquito transmission of P. cynomolgi.*—This work was continued intensively during April, by the end of which month it was possible to infect monkeys by bites from as many as 350 mosquitoes each, 88 per cent of which had been infected.

The experiments designed to localize sporozoites in the skin and spleen were completed as far as possible, but some at least will have to be repeated.

(ii) *Pathological work.*—Tissues from 136 infected monkeys are being subjected to detailed examination, designed to cover all phases of the parasite's development.

In the spleen 50 per cent of the animals failed to develop infections: in the skin 39 per cent. There appears to be a correlation between the infectivity of various batches of mosquitoes and the time at which they developed.

In the spleen, so far, no trace has been found in smears of sections either of sporozoites or of their progeny, from 12 hours to 12 days after inoculation.

In the skin, where it was calculated in one case that any high power field of inoculum ought to show over 1,000 sporozoites, only about 3 sporozoites were discovered in 15 biopsies made one hour after inoculation, the monkey eventually developing malaria.

(iii) *Tissue culture.*—By a simple technique, it has been found possible to grow histiocytes, fibroblasts and lymphocytes from monkey spleen without difficulty. Cultures of the liver, however, yield only a very few of these cells; while those of the brain show death of the capillary endothelium and growth of histiocytes, fibroblasts and lymphocytes from the adventitial cells and from the pia mater. No growth of malaria parasites has been obtained.

PROPOSALS FOR 1947–48.

The completion of investigations now in progress and the detailed examination of the mass of material which has been accumulated during the current year.

3. *Enquiry on adult A. stephensi under the Director, School of Tropical Medicine, Calcutta.*

(i) Extensive studies have been carried out regarding the daytime resting places of *A. stephensi.*

(ii) An attempt has been made to determine the proportions of the different races of *A. stephensi* present in Calcutta.

(iii) A considerable amount of material has been collected for determination of the source of blood meals of *A. stephensi* by the precipitin test.

(iv) Investigations have been undertaken as regards the seasonal prevalence and density of *A. stephensi* in Calcutta.

(v) Several hundred specimens of *A. stephensi* have been dissected, but with negative results.

(vi) Investigations have been carried out which suggest that *A. stephensi* in Calcutta has a wide range of dispersal.

PROPOSALS FOR 1947–48.

(i) The search for new daytime resting places, particularly houses without the influence of bovine attraction, will be continued. Observations on microclimate are contemplated.

(ii) Studies on seasonal and numerical prevalence will be continued for another year. It is proposed to test by experiment the influence of bovine

attraction on numbers of adult mosquitoes in a particular catching station. The experiment will be supported by observations made pari passu on the composition of the catches and probably also on the source of the blood meal, particularly when there is no bovine blood at hand.

(iii) The study of other aspects of adult habits, such as mating in relation to feeding, nocturnal movements, etc., will be taken up as time and opportunity permit.

4. *Enquiry on the control of sandflies by spraying DDT under Dr. D. N. Roy.*

Owing to local disturbances and other reasons very little work has been possible, the investigation so far undertaken being of a preliminary nature only.

PROPOSALS FOR 1947-48.

A continuation of the line of work laid down for the current year.

II. PROPOSALS FOR NEW ENQUIRIES.

1. *Enquiry on the properties of pyrethrum flowers by Dr. D. N. Roy.*

It is proposed to undertake researches regarding the active principles and chemical and biological properties of pyrethrum flowers with special reference to their deterioration after storage for prolonged periods.

2. *Enquiry on the chemotherapy of malaria under Dr. R. N. Chaudhuri.*

Chemotherapeutic investigations on (a) Paludrine and (b) CAM-AQI in the treatment of malaria.

To study the effect of these drugs on malaria and to determine the minimum effective dose.

To make observations on concomitant features of malaria or side effects, if any.

To determine the effect of these drugs on the viability of the gametocytes in mosquito.

3. *Enquiry into the epidemiology of malaria in Singur area with particular reference to the problem of transmission under Drs. R.B. Lal, S. C. Seal and L. M. Bhattacharji.*

(i) The study of the incidence and density of breeding of *A. philippinensis* and other species in this area.

(ii) The study of the flora, fauna, and physical and chemical composition of water of mosquito breeding places.

(iii) The study of the numerical prevalence, density and infectivity of different species of adult mosquitoes.

(iv) Examination of the blood of new born babies twice each month to determine the period of malaria transmission.

(v) Determination of spleen and parasite rates in children and adults month by month.

(vi) The study of the seasonal output of gametocytes in infants, children and adults.

(vii) The study of data regarding the prevalence of malaria and of metereological conditions.

III. The Committee made the following recommendations :—

EXISTING ENQUIRIES

1. Insecticide and Mosquito Repellent Enquiry (under the Director, Malaria Institute of India, Delhi).
2. Mammalian Malaria Enquiry (under the Director, C.R.I., Kasauli).
3. Enquiry on adult *A. stephensi* (under the Director, School of Tropical Medicine, Calcutta).
4. Enquiry on the control of sandflies *P. argentipes* by spraying with DDT (under Dr. D. N. Roy at the School of Tropical Medicine, Calcutta).

The Committee approved the proposals put forward for the continuance of these enquiries in 1947-48.

IV. PROPOSALS FOR NEW ENQUIRIES.

1. Enquiry on the active principles of pyrethrum flowers, their chemical and biological properties and their deterioration due to storage (under Dr. D. N. Roy, School of Tropical Medicine, Calcutta).

The Committee did not recommend this proposal.

2. Enquiry on malaria (under Dr. R. N. Chaudhuri, School of Tropical Medicine, Calcutta).

The Committee approved this proposal, after noting that the investigation will be confined to human malaria cases only.

3. Enquiry into the epidemiology of malaria in the Singur area with particular reference to the problem of transmission (under Drs. R. B. Lal, S. C. Seal and L. M. Bhattacharji, All-India Institute of Hygiene and Public Health, Calcutta).

The Committee approved the proposal put forward for this enquiry, but recommended that the investigation be undertaken in hyperendemic villages. It was pointed out in the course of discussion that a number of such villages exist within a distance of about twelve miles from Singur.

V. The Committee passed the following resolution :—

Having regard to the grave loss of man-power caused by malaria among railway employees in India and the fact that a number of officers and other personnel with several years' field experience of malaria control in the army are now available for employment, the Committee recommends that the Railway Board be urged to take advantage of this circumstance to set up an adequate antimalaria organisation for every railway which operates in areas where the disease is prevalent.

VI. At the conclusion of the meeting Lieut.-Colonel Afridi informed the Committee that Major-General Sir Gordon Covell, C.I.E., K.H.P., I.M.S., will be leaving India shortly on retirement.

The Committee then recorded their deep appreciation of the most valuable services rendered by Sir Gordon to the cause of malaria. They felt that it was due entirely to his unceasing work and efforts that the administrators and the people of India have become malaria-conscious and that it was due to his unflagging zeal, energy and guidance that the standard of malaria investigation and control has been raised to its present high level.

The Committee felt that Major-General Sir Gordon Covell's departure from India would be a loss not only of a pioneer research worker but a very dear friend of the country. They wished him and Lady Covell " Bon-Voyage " and a long and happy retirement.

VII. The Committee also recorded its appreciation of the malaria research work done during his long stay in this country by Major R. Senior White, Malariologist, Bengal-Nagpur Railway. They hoped that Major and Mrs. Senior White will have many happy days at home after their long and useful service in India.

REPORT OF THE FOURTEENTH MEETING OF THE NUTRITION ADVISORY COMMITTEE OF THE INDIAN RESEARCH FUND ASSOCIATION HELD IN NEW DELHI, ON THE 21st JULY, 1946.

COMPOSITION OF THE COMMITTEE.

PRESENT.

Colonel E. Cotter, C.I.E., V.H.S., I.M.S., Public Health Commissioner with the Government of India. (*Chairman*).

Professor S. P. Agharkar, Professor of Botany and Head of the Botany Department, University College of Science, Calcutta.

Dr. K. P. Basu, Reader in Chemistry and In-charge of the Biochemical Department, University of Dacca, Dacca.

Dr. V. N. Patwardhan, 774, Parsi Colony, Dadar, Bombay.

Dr. D. M. Sen, Offg. Secretary to the Government of India, Department of Education.

Mr. Grahame Williamson, O.B.E., Animal Husbandry Commissioner with the Government of India.

Dr. Bashir Ahmad, Assistant Director, Council of Scientific & Industrial Research, University Buildings, Delhi. (*Secretary*).

Dr. B. C. Guha, Sir Theodore Gregory, Lieut.-Colonel C. M. Nicol, Dr. G. Sankaran and Sir Datar Singh were unable to attend.

The following were present by invitation :—

Dr. S. Ahmad, Nutrition Officer, Public Health Department, Bankipur, Patna.

Dr. (Miss) Kamala Bhagvat, Assistant Director, Nutrition Research Laboratories, Coonoor.

Major P. D. Bhave, Officer on Special Duty (Nutrition), Central Provinces & Berar.

Dr. R. E. Dadachanji, Nutrition Officer, Finance Department (Supply), Haffkine Institute, Bombay.

Dr. M. B. Daver, Nutrition Officer, Medical and Public Health Department, Hyderabad (Deccan).

Dr. P. V. George, Nutrition Officer, Public Health Department, Madras.

Mr. T. Gonsalves, Assistant Secretary, Indian Research Fund Association.

Major B. N. Khan, I.M.S., Chief Health Officer, Delhi Province, Delhi.

Dr. V. R. Khanolkar, Director of Laboratories, Tata Memorial Hospital, Bombay.

Mr. W. H. Kirby, Rationing Adviser to the Government of India, Department of Food.

Dr. K. Mitra, Deputy Director (Rationing), Government of India, Department of Food.

Dr. M. V. Radhakrishna Rao, Assistant Director, Department of Nutrition and Experimental Pathology, Haffkine Institute, Bombay.

Lieut.-Colonel G. Verghese, I.M.S.(R), Director of Medical and Health Department, Tata Iron & Steel Co., Ltd., Jamshedpur.

Major O. P. Verma, Assistant Director (Nutrition), Medical Directorate.

(I) THE PROGRESS OF NUTRITION WORK IN PROVINCES AND STATES.

The Committee considered reports of work carried out in Bihar, Punjab, Central Provinces, Bombay, Madras and Hyderabad (Deccan). A brief account of the work is given below :

(a) *Bihar* :—Field work including dietary and nutrition surveys have been continued. A survey of the diet of 80 high caste Hindu families consisting of 622 persons has been undertaken and the nutrition survey of 498 children in rural areas near Mokamah has been carried out. Laboratory work has included analysis of cooked food. Extensive publicity and nutrition education work has been undertaken at the All India Industrial Exhibition at Patna, in Hijla Mela in Dumka, at Yagoda Sat Sangh at Ranchi and at the All India Swadeshi Exhibition, Patna. The exhibits, consisted of popular charts, posters, models and display of balanced diets in trays. Leaflets were distributed and popular lectures given. Preliminary arrangements were under way with respect to a milk distribution scheme for school children.

(b) *Punjab* :—The nutrition officer, Dr. M. Musa Khan, has been on study leave since November, 1945. The nutrition staff in the province has been augmented. A nutrition and diet survey in the Ambala district has just been completed and the data collected were being worked out. The experiment on the distribution of iodized salts to the inhabitants of certain villages in the Kangra Valley with the object of determining its effect on the incidence of goitre will have been going for 5 years in March, 1947, when its results will be finally assessed. From observations so far made there is evidence of a beneficial effect on the incidence of this disease. Government was considering a proposal to establish a factory for the iodisation of salt in the Kangra valley so that all the inhabitants of the valley may have the reinforced product.

Rationing of wheat and sugar which has been in force in the 3 towns of Lahore, Amritsar and Rawalpindi, does not appear to have had any detrimental effect on the health of the population.

The routine work with regard to nutrition publicity, and advice to hospitals and institutions regarding diets has been continued. District Health Officers continued to submit the quarterly reports regarding the general state of nutrition and the availability of food supplies in the districts and no deterioration in either has so far been reported.

While discussing this report the Committee considered that routine general reports on the state of nutrition are of limited value. An estimate of the deterioration or otherwise of health can only be obtained by a rapid survey based on objective methods. These were discussed fully at the Ninth meeting of the Nutrition Advisory Committee held on the 1st and 2nd October, 1943, and methods of such surveys are given in the report of the meeting. Further, the maintenance of up-to-date vital statistics particularly those of mortality by sex and by age groups is essential if the health authorities are to keep aware of any deterioration in the public health.

(c) *Central Provinces* :—Work on nutrition and diet surveys has been continued. During this year diet surveys have been carried out among the aboriginal tribes. Arrangements are being made for starting school meals in some of the primary schools in Nagpur. Industrial organisations were being encouraged to start canteens for their workers and routine work on publicity and propaganda was being carried out.

Work of particular interest includes investigations on lathyrism, the incidence of which has increased during recent years when due to food shortage *Lathyrus sativus* (Teora or Teori) has been the main source of subsistence of some of the poorer sections of the population. Extensive field investigations in 6 districts showed the presence of 7,413 cases of lathyrism. The poorer sections of the populations affected were living largely and in some instances exclusively on Teora, the average consumption being 15·4 oz.

It was reported by Dr. K. P. Basu that in his laboratory a diet consisting of 30% of *Lathyrus sativus* resulted in the appearance of symptoms such as diarrhoea, weakness, loss of weight and nodules on the legs in pigeons even in the presence of sufficient amounts of Vitamin B complex; Vitamin A appeared to exert a slightly beneficial effect. Work at Coonoor was undertaken on guinea pigs by incorporating this pulse at 30%, 50% and 75% level of feeding and symptoms such as loss of hair, dermatitis and ulcers on hind legs appeared in most of the animals. Vitamin B_1 seemed to have no effect while inclusion or exclusion of husk made no difference on the appearance of the symptoms.

The Committee was of the opinion that the pathological material now available in this province should be collected and investigated. It was decided that a comprehensive scheme for this investigation should be drawn up by a joint Sub-Committee of the Nutrition Advisory Committee and the Clinical Research Advisory Committee.

(d) *Bombay* :—The Government of Bombay has continued the excellent work on the problem of providing better nutrition for its population and the Committee paid a tribute to this work. The work has been continued on lines mentioned in the last report. The lines of particular interest undertaken lately are : (i) the supply of groundnut cake atta at the rate of 1 anna per lb. which could be mixed with ordinary wheat atta to the extent of 15 to 20% for making chapattis. This product was slowly finding friendly reception among the educated section of the public, (ii) the milk scheme was being continued with success and was now being extended to Poona city and suburbs ; (iii) a scheme for catching deep water sharks by power propelled vessels has been sanctioned and great increase in shark liver oil production was expected as soon as the scheme comes into operation. Vitamin A concentrates from shark liver oil were being prepared in the Haffkine Institute in a quantity enough to yield as many as 4,000,000 tablets per month ; (iv) Government vegetable shops were being maintained in 12 big municipal markets of the city and the Government was thus keeping an indirect control on the price of vegetables ; (v) a potato scheme had been put into operation by which potatoes were sold to the public through the 245 Government grainshops at rates varying from 2 to 4 annas per lb. Up to the end of June, 1946, 68,41,000 lb. of potatoes have been sold through Governmental agencies ; (vi) further extensions of community feeding and canteens in industry have been made and the industrial canteens now number 304. Work on school feeding and institutional feeding reported in the last year's report was also being continued ; (vii) further survey of diet and nutrition has been undertaken and work on publicity and education has been carried on.

(e) *Madras* :—Work in Madras has included studies of diets and deficiency diseases ; relief measures such as free mid-day meals for school children ; free milk supply to priority consumers ; administration of yeast to school children and industrial canteens ; studies of vital statistics in relation to food shortage

and nutrition education. It was mentioned by the nutrition officers that in a group of children, 10% of whom had stomatitis, the disease disappeared on their being fed by yeast in spite of the fact that the dose was as small as 45 grains per child per day.

(f) *Hyderabad (Deccan)* :—The mobile canteen scheme is now in operation. Each canteen included a demonstration kitchen and balanced nutritious meals were being supplied to public at cost price. Work had been carried out on the use of groundnut cake flour. It is found that when 10% is mixed with ordinary wheat flour the baking qualities are not affected and even when it is mixed to the extent of 30%, chapattis can be made. Its use is being recommended to the public in the homes as well as in public bakeries.

(II) Consideration of the report of the Food Fortification Sub-Committee of the Nutrition Advisory Committee.

The report by this Sub-Committee was considered and was accepted with the following modifications.

The paragraph on "Food Yeast" was deleted. The Committee decided that this question should be considered in detail at the next meeting of the Nutrition Advisory Committee.

The Report of the Sub-Committee on Food Fortification as adopted by the Nutrition Advisory Committee is given as *Appendix* I to this report.

(III) Discussion of a letter from the Secretary, Imperial Council of Agricultural Research, New Delhi, regarding work on mango seed kernel conducted at the Nutrition Section of the Imperial Veterinary Research Institute, Izatnagar.

After some discussion the Committee agreed that while mango seed kernel might be suitable for feeding live-stock, its use as human food could not be recommended as a practical proposition.

(IV) Consideration of the following notes on vegetable milks.

(i) "Digestibility of soya milk" by N. Krishnaswamy, S. S. De and V. Subrahmanyan of the Department of Biochemistry, Indian Institute of Science, Bangalore.

(ii) "Nutritive value of ground-nut milk" by H. S. R. Desikachar, S. S. De and V. Subrahmanyan of the Department of Biochemistry, Indian Institute of Science, Bangalore.

(iii) "A practical approach to the milk problem in India" (vetegable milks—their nutritive value and ultimate practical possibilities) by S. S. De, H. S. R. Desikachar and V. Subrahmanyan of the Department of Biochemistry, Indian Institute of Science, Bangalore.

Since Dr. V. Subrahmanyan could not be present at this meeting, the consideration of these notes was postponed to the next meeting of the Committee.

(V) Consideration of a note on the nutritive value of Vanaspati or Hydrogenated Oils by Dr. Bashir Ahmad.

The Committee considered the above note and accepted it with slight modifications.

The note as accepted is printed as *Appendix* II to this Report.

APPENDIX I.

Report of the meeting of the Food Fortification Sub-Committee of the Nutrition Advisory Committee held on the 20th July, 1946.

PRESENT.

1. Dr. K. P. Basu.
2. Dr. (Miss) Kamala Bhagvat.
3. Dr. V. N. Patwardhan.
4. Dr. Bashir Ahmad (Secretary).

Major B. N. Khan, Dr. R. E. Dadachanji and Dr. M. V. Radhakrishna Rao also attended.

A note on the fortification of staple foodstuffs by Dr. Bashir Ahmad was considered and the Sub-Committee reached the following conclusions:

(I) FORTIFICATION OF CEREALS.

The Indian diets which are largely based on cereals are generally deficient in calcium and vitamins. The Sub-Committee was strongly of the opinion that it was essential to fortify the staple cereals. The Sub-Committee made the following recommendations:—

(i) *Wheat*:—Wheat atta or flour produced in the country or imported should not be below 95% extraction, preferably the extraction should be 90% or above. For fortification with calcium, calcium carbonate should be used in the proportion of one part for 640 parts of flour. Calcium carbonate used should be of the B.P. quality and particularly free from flourine, arsenic and lead. The addition of this quantity of calcium carbonate will not make the flour in any way injurious or unwholesome but will definitely add to its nutritive value. The addition of calcium carbonate should be made compulsory in large mills where atta is produced. In rationed areas the appropriate amount of calcium carbonate may be separately given to the consumers along with their ration of wheat grains. In view of the prejudice against the use of fortifying agents, extensive education for the necessity of using calcium carbonate in flour should be undertaken.

Under the present conditions and at the extraction level recommended, fortification of wheat and wheat flour with thiamin, niacin and riboflavin may not be undertaken at present.

(ii) *Rice*:—The Sub-Committee discussed in general the work which has already been undertaken with regard to the fortification of this cereal, including the Huzenlaub conversion and the Roche fortification process, and the conclusions which had already been arrived at by a joint meeting of this Sub-Committee and the Committee of the Food Department. The Sub-Committee was of the opinion that further work should be undertaken on the rice problem, and standard conditions for parboiling with known varieties of paddy worked out. On the basis of this work recommendations should be made to the Government to design small units of parboiling plants suitable for use in villages.

During this investigation the question of fortifying rice with calcium salts during parboiling should be borne in mind. It was noted that some work along this line has already been done in some laboratories particularly by Dr. Sanjivarao at Bangalore.

(iii) *Millets such as Ragi, Jowar and Bajra :*—Since these are consumed whole, the Committee did not consider their fortification at present necessary. Later on if further work on the deficiencies and the possibilities of fortification is forthcoming the question may be considered.

(II) Fortification of edible fats.

(i) *Hydrogenated Vegetable Fat :*—The Sub-Committee felt that it was desirable to fortify all hydrogenated fats with vitamin A and vitamin D. Some hydrogenated fats sold in the market are labelled " contains vitamins ". Such labels are misleading since only vitamin D has been added, while vitamin A, the more important vitamin A of butter, is absent. Fortification with synthetic vitamin D is easy but there are difficulties of fortification of these fats with vitamin A under the Indian conditions. Since margarine was being fortified with vitamin A in western countries, it should be possible to find ways and means of fortifying hydrogenated vegetable fats in this country. The Sub-Committee recommended that work should be undertaken on this subject in the various laboratories in the country and the preparation of vitamin concentrates from shark liver oil by molecular distillation should be undertaken.

(ii) *Vegetable Oils :*—The Committee felt that while it will be desirable to fortify vegetable oils with vitamins A and D particularly for people subsisting on vegetable oils as the sole source of fats, the fortification of vegetable oils need not at present be attempted in view of the cooking habits of the people. Work may be continued on the loss of nutritive factors from vegetable oils in cooking and on the development of such cooking methods as will minimise these losses.

(III) Shark liver oil.

The Sub-Committee recommended that the shark liver oil industry and the production of vitamin A concentrates from shark liver oil, particularly by molecular distillation, should be encouraged.

(IV) Synthetic vitamins.

In the opinion of the Sub-Committee the production of synthetic vitamins in the country is exceedingly important for fortification of foodstuffs and for supplementing the diets. It was recommended that immediate steps should be taken by the Government for their large-scale production in India.

(V) Education and publicity.

Experience has shown that generally a great deal of opposition arises from any interference with the common foodstuffs of the people. Success of the entire programme of fortification of staple foods depends upon education of the people along right lines. Education is, therefore, an essential preliminary for any policy of large-scale fortification of foodstuffs.

APPENDIX II.

A Note on the Nutritive Value of Vanaspati or Hydrogenated Vegetable Oils by Dr. Bashir Ahmad, M.Sc., Ph.D. (London), F.N.I.

During the last quarter of a century, Vanaspati has as an edible fat rapidly gained greater and greater popularity in this country and its nutritive value has been the subject of considerable comment and discussion. It has been lately the subject of discussion by Committees of the Imperial Council of Agricultural Research; and the Hyrogenated Oils Committee of the Technical Panel of the Food Department. A symposium was also held on this subject at the last session of the Indian Science Congress at Bangalore.

The comments and recommendations of the various bodies mentioned above, who have discussed this problem are briefly summarised below.

1. Vanaspati is more or less devoid of vitamins A and D. In spite of this as a fat it has good nutritional value, but it cannot be considered as a substitute for ghee.

2. Suggestions have been made that the consumption of vanaspati is injurious to health causing particularly irritation to the throat and respiratory passages. Even medical opinion has sometimes seemed to countenance such reports but there is so far no unequivocal scientific evidence that vanaspati is unwholesome.

3. Such experimental investigations as have been undertaken, show that its digestibility and absorption are not very much inferior to vegetable oils, particularly when its melting point is not very much higher than the body temperature. There is some scientific evidence to show that when colonies of rats are fed on hydrogenated fats for a number of generations, they have somewhat poor rates of growth in 2nd and 3rd generation; poor reproductive ability; and sometimes loss of fur. Other experiments have indicated that its consumption affects somewhat adversely the utilisation of other nutritional factors such as vitamins and minerals. It is generally agreed, however, that such scientific evidence will have to be based on very much more extended experiments before it can be accepted, that vanaspati really has a deleterious effect upon health after long periods of consumption.

4. The relative nutritive value of deodorized refined vegetable oils appears to be slightly better than that of the hydrogenated product. The hydrogenated product sometimes has high melting points, particularly when the product is manufactured in summer, and the digestibility and absorption of such high melting products is poorer.

5. It was recommended by the Hydrogenated Oils Committee of the Technical Panel of the Food Department that

(a) the melting point of the vanaspati produced in the country should not exceed 38°C. since as the melting point increases the digestibility and absorption decreases;

(b) researches should be encouraged on the nutritive value of vansapati to obtain further information on the subject. These researches should include the study of the effect of long range feeding on growth and

well being; the stability of vitamins in fortified vanaspati; the amount of vitamin E present and fortification of vanaspati with vitamin E for increasing its value as an antioxident, or for the utilisation of other vitamins; and the comparative nutritive value of the different varieties of hydrogenated oils.

The above comments show that while some investigations have been made on this important subject chiefly under the auspices of the Imperial Council of Agricultural Research further work is necessary before a definite opinion can be expressed regarding the nutritive value of hydrogenated vegetable oils. It seems desirable that the Indian Research Fund Association should also encourage researches on the nutritive value of vanaspati, a subject on which so far there is inadequate scientific information, and which continues to agitate the public mind in view of conflicting and disturbing opinions.

REPORT OF THE FIFTEENTH MEETING OF THE NUTRITION ADVISORY COMMITTEE OF THE INDIAN RESEARCH FUND ASSOCIATION HELD IN BOMBAY ON THE 9th DECEMBER, 1946.

COMPOSITION OF THE COMMITTEE.

PRESENT :

Colonel E. Cotter, C.I.E., V.H.S., I.M.S., Public Health Commissioner with the Government of India. (*Chairman*).

Professor S. P. Agharkar, Professor of Botany & Head of the Botany Department, University College of Science, Calcutta.

Dr. Bashir Ahmad, Assistant Director, Council of Scientific & Industrial Research, New Delhi.

Dr. K. P. Basu, Dairy Chemist, Imperial Dairy Research Institute, Bangalore.

Dr. M. V. Radhakrishna Rao, Assistant Director, Department of Nutrition and Experimental Pathology, Haffkine Institute, Bombay.

Mr. Grahame Williamson, O.B.E., Animal Husbandry Commissioner with the Government of India, New Delhi.

Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor. (*Secretary*).

Dr. B. N. Adarkar, Dr. B. C. Guha, Lieut.-Colonel C. M. Nicol, Dr. G. Sankaran, and Sir John Sargent were unable to attend.

The following were present by invitation :—

Dr. (Miss) Kamala Bhagvat, Assistant Director, Nutrition Research Laboratories, Coonoor.

Major P. D. Bhave, Officer on Special Duty (Nutrition), Central Provinces and Berar.

Professor B. V. Bhide, Maharajah Pratap Sinh Chemical Laboratory, S. P. College, Poona.

Dr. R. G. Chitre, Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S. (R), Director, Drug Research Laboratory, Jammu, Kashmir.

Dr. R. E. Dadachanji, Nutrition Officer, Finance Department (Supply), Haffkine Institute, Bombay.

Mr. N. C. Datta, Grant Medical College, Bombay.

Dr. M. B. Daver, Nutrition Officer, Medical and Public Health Department, Hyderabad (Deccan).

Dr. S. S. De, Lecturer on Food Technology, Indian Institute of Science, Bangalore.

Mr. T. Gonsalves, Assistant Secretary, Indian Research Fund Association.

Dr. A. N. Goyle, Professor of Pathology, King Edward Medical College, Lahore.

Dr. M. O. T. Iyengar, Entomologist, Bengal Public Health Department, Calcutta.

Major M. Jaffar, I.M.S., Director of Public Health, Bengal, Calcutta.

Major B. N. Khan, I.M.S., Chief Health Officer, Delhi Province, New Delhi.

Dr. Mohd. Musa Khan, Nutrition Officer, Public Health Department, Punjab, Lahore.

Mr. W. H. Kirby, O.B.E., Rationing Adviser to the Government of India, Department of Food, New Delhi.

Dr. K. V. Krishnan, Professor of Microbiology, All-India Institute of Hygiene and Public Health, Calcutta.

Lieut.-Colonel C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene and Public Health, Caclutta.

Dr. Jivraj N. Mehta, Khambatta Hill, 16, Altamount Road, Bombay.

Lieut.-Colonel C. L. Pasricha, I.M.S., Director, School of Tropical Medicine, Calcutta.

Major T. C. Puri, I.M.S., Deputy Public Health Commissioner with the Government of India, New Delhi.

Mr. K. K. P. Narsingha Rao, Assistant Director of Nutrition, Government of India, Department of Food, New Delhi.

Professor V. Subrahmanyan, Professor of Biochemistry, Indian Institute of Science, Bangalore.

Major O. P. Verma, Deputy Director (Nutrition), Medical Directorate, New Delhi.

(I) To review work in progress and consider the question of continuation or otherwise of existing enquiries.

(A) The Committee recommended the continuation of the following enquiries :—

(i) Nutrition researches at the Nutrition Research Laboratories, Coonoor.

(ii) Additional nutrition enquiry under the Director, Nutrition Research Laboratories, Coonoor.

(iii) Nutrition research unit under Dr. R. G. Chitre at the Seth G.S. Medical College, Bombay.

(iv) Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras.

(v) Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary protein to blood and muscle haemoglobin under Prof. M. Damodaran at the University Biochemical Laboratory, Triplicane, Madras.

(vi) Enquiry on the nutritive value of soya bean milk under Dr. S. S. De at the Indian Institute of Science, Bangalore.

(vii) Ascorbic acid enquiry under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta. The Committee recommended that this enquiry be continued provided a detailed programme of work for a limited period is submitted and personal supervision in Calcutta by Dr. B. C. Guha is assured.

(viii) Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta. The Committee expressed disappointment at the progress of work done on this enquiry during the year under review and recommended that the enquiry be recommended for continuation provided an assurance for personal supervision in Calcutta from Dr. B. C. Guha was forthcoming.

(ix) Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay. Mr. Datta had contemplated closing down this enquiry but after discussion with him the Committee requested that he should put up a revised budget for continuing the enquiry.

(x) Nutrition research unit under Dr. K. P. Basu at Dacca University, Dacca. The Committee noted that Dr. Basu has left Dacca for Bangalore and that the nutrition research unit has been working under Dr. H. N. De since Dr. Basu's departure. The Committee recommended the continuation of the unit at Dacca under Dr. H. N. De.

(xi) Enquiry entitled "Preparation of anti-rachitic substances" under Prof. B. V. Bhide at the Maharajah Pratap Sinh Chemical Laboratory, S. P. College, Poona. The Committee recommended the continuation of the enquiry. The Committee further recommended that a non-recurring grant of Rs. 5,000/- should be made for the purchase of high pressure hydrogenation equipment for this enquiry.

(B) The following enquiry is terminating this year:—

Enquiry into estimation of vitamin A activity of plant foods under Dr. Bahsir Ahmad at the Laboratories of the Council of Scientific & Industrial Research, University Buildings, Delhi.

(2) To consider new proposals.

(A) The Committee recommended the following proposals for new enquiries:—

(i) Enquiry on plant carotenoids in relation to their biological importance under Dr. Bashir Ahmad at the Laboratories of the Council of Scientific & Industrial Research, University Buildings, Delhi.

(ii) Enquiry on the growth promoting factor present in Bengal gram under Dr. Bashir Ahmad at the Laboratories of the Council of Scientific & Industrial Research, Delhi.

(iii) Bangalore school feeding scheme under Prof. V. Subrahmanyan, Professor of Biochemistry, Indian Institute of Science, Bangalore. The Committee recommended that the scheme should be worked in close collaboration with the Director, Nutrition Research Laboratories, Coonoor, who will provide the medical officer to supervise the working of the scheme and assessment of the results.

(iv) Nutrition research unit under Professor V. Subrahmanyan, Professor of Biochemistry, Indian Institute of Science, Bangalore. The Committee accepted in principle the desirability of establishing a nutrition research unit at the Indian Institute of Science, Bangalore, under

Prof. V. Subrahmanyan. The Committee requested Prof. Subrahmanyan to recast his programme of work in order to conform more closely with the lines of nutrition research being followed by the Nutrition Advisory Committee. The programme of work, which will be received later, will be considered again by the Nutrition Advisory Committee at their next meeting.

(B) The following enquiries were not recommended:—

(i) Nutrition research unit under Dr. B. C. Guha at the University College of Science & Technology, Calcutta. The Committee was of the opinion that, in view of what has been said about the existing enquiries under Dr. Guha (at present supervised by Dr. Ghosh), it is premature to consider the establishment of a nutrition research unit at Calcutta under Dr. Guha.

(ii) Enquiry on the metabolism of manganese under Prof. M. N. Rudra, Professor of Medical Chemistry, Darbhanga Medical College, Laheriasarai.

(iii) Enquiry to investigate the money spent on food and cooking fuel and the nature of the food consumed in 300-500 households in a stated geographical area and during a limited period under Miss S. Ram, Consulting and Analytical Chemist, 49, Hanuman Road, New Delhi.

(3) To consider the progress of nutrition work in provinces and states.

Punjab.

The Chairman informed the Committee that he had received a letter from the Director of Public Health, Punjab, regarding the views expressed by the Committee at their last meeting on the limited value of routine general reports on the state of nutrition and on the necessity of maintaining up-to-date vital statistics. These views had been unfortunately included under the description of work carried out in the Punjab in the last report of the Nutrition Advisory Committee. The Committee recorded that their views regarding the limited value of routine general reports and the necessity of maintaining up-to-date vital statistics were in no way meant to apply to the Punjab in particular, but that the discussion referred to India generally and in particular to the provinces threatened with famine of which the Punjab, certainly, was not one.

Central Provinces & Berar.

Lathyrism:—During the present year a constant and vigilant watch on fresh cases of Lathyrism has been kept. Civil Surgeons have been requested to submit monthly reports regarding the number of fresh cases. Steps have been taken to prevent the spread of the disease. Measures taken in this connection include (i) making broken rice available in the affected villages at a cheap rate, (ii) the enforcement of the "Teora Control Order of 1946" which lays down that Teora will not be sold or otherwise disposed of in any form, except in the form of a mixture with other specified grains, viz., wheat, gram, juar, rice, etc., in a proportion of not more than one-third of the total quantity of food grains, and (iii) Government propaganda to restrict the cultivation of Teora and to

grow other rabi crops in its place. It is hoped that research into the histopathological, biochemical and clinical cum nutritional aspect of lathyrism will be started during the coming year.

Improvement of aboriginal diets :—Free distribution of seeds has been started among the aboriginals with the object of making up the deficiencies in their ill-balanced diets.

Propaganda and education :—Two press notes have been issued on (a) " The School Feeding Schemes " and (b) " Are you wasting good food ? " which describe the nutritional superiority of parboiled rice over raw rice and how it would help in the present food shortage if parboiled rice were consumed.

School feeding schemes have just been started in primary schools in Nagpur and the result of these experiments will be reported later.

Bihar.

The work done in Bihar during 1946 included a dietary survey of food intake of caste Hindus in the Districts of Saran and Patna. A survey of non-caste Hindus was also carried out in the district of Patna. The non-caste Hindu families consisted of agricultural labourers, some of them having no land. The children of the villages in which dietary surveys were carried out were also examined clinically. The nutritional state of caste Hindus was found to be satisfactory while that of low caste Hindus was very unsatisfactory.

The experiment on the feeding of food yeast to children is in progress and the results will be reported later.

Education and publicity :—Education and publicity work was continued and at various exhibitions popular charts were exhibited showing different types of balanced diets. Popular lectures were given by the Nutrition Officers and other officers of the Public Health Department. Lectures on food and nutrition were also delivered to candidates undergoing Sanitary Inspector's training.

Hyderabad State.

School feeding schemes :—A start has been made with primary schools where 12,000 children are being fed. The cost of this scheme is over 3½ lakhs and the whole cost is being borne by the Government. The feeding of the worker which came to the forefront during the war continues to be one of the main plans of labour welfare and the idea behind the setting up of mobile units and canteens is to supply the deficiency in the diet of the worker. Many employers have appreciated the long range advantages of the seemingly costly step of opening canteens in their industries, but as a class they have been slow to grasp the value of the indirect gain to them in opening these canteens, which will result in higher efficiency and more loyalty. The Government have started a mobile canteen scheme with the aim of providing wholesome, balanced and hygienically cooked food at reasonable prices.

Nutrition restaurants :—Five nutrition restaurants in Hyderabad City and one in Warangal have been sanctioned. In these restaurants, the public will get cheap, nutritious and balanced meals at a price which the average person can afford.

Seed and manure distribution :—The distribution of seed and manure has been taken in hand and the result has been an increase in food production amounting to 16,000 tons.

Fisheries :—The Hyderabad Nutrition Committee have recommended to the Government the development of fisheries. The food values of twenty varieties of fish have been ascertained and further work is in progress.

Repeat nutrition surveys :—Repeated diet and nutrition surveys in the rural areas have confirmed early findings that there is a definite decrease in the intake of articles of diet and an increase in pre-clinical and clinical signs of deficiency diseases.

Publicity :—Every opportunity is taken to give demonstrations by magic lanterns and cinema cars in schools and villages. The nutrition staff are specially deputed for this work. Public lectures in nutrition and diet are given. Lectures to nurses and health visitors were also arranged and a nutrition exhibition was held.

Madras.

Work done during the year included studies on diets and deficiency diseases in various parts of the province with special reference to certain of the deficit districts such as, Anantapur, Bellary, Kurnool and Ramnad. Among the children examined during the surveys, unusual prevalence of any specific deficiency conditions was not observed in any area. Out of about 1,200 children examined, only 37 cases of Vitamin A deficiency, 16 cases of Vitamin B_2 deficiency and one case of Vitamin C deficiency have been recorded, though about 70 cases have been classified as showing signs of sub-nutrition.

Special relief measures :—Special relief measures included the supply of free midday meals to school children. This is linked up with the scheme of compulsory primary education in the province and the scheme is administered jointly by the Department of Education and the local authorities. During the year about 7,500 school children were supplied with free midday meals in the City of Madras under the auspices of the Corporation. School children in all labour schools under the Government are provided with free midday meals. Milk is distributed free to infants, toddlers, expectant and nursing mothers. In the City of Madras about 2,000 measures of milk are supplied each day by Government. The free supply of milk is restricted to families whose income is below Rs. 50 p.m. Government have also sanctioned a scheme of free distribution of milk reconstituted from imported milk powder to all school children attending standards I to V, excepting those who are already receiving midday meals in the districts of Anantapur, Bellary, Salem, Chittoor and North Arcot.

Industrial canteens :—There are now 227 canteens attached to industrial establishments in the province.

Educative measures on 'Food and Nutrition' :—The Central Bureau of Nutrition at Madras is being equipped for undertaking general measures of propaganda on matters of food and nutrition in the province. Full use is being made of cinema films, suitable pamphlets published in local languages, lectures and radio broadcasts to educate the public.

"Grow more food" campaign—Production of compost manure from wastes :—Useful work has been done on the "Grow more food" campaign and in particular on the production of compost manure from wastes.

A "Grow more fish" campaign has been vigorously launched and experiments are also in progress at the Fisheries Technological Station to improve

existing methods of curing fish so as to retain its nutritive value to a maximum. The Department of Industries have a programme to produce in large quantities and to distribute shark liver oil among priority consumers through hospitals and Maternity & Child Welfare Centres.

Studies on vital statistics in relation to food shortage :—The figures for the first 8 months of 1946 have been compared with the corresponding date for 1944 and 1945 and also with a ten year average for the period 1932-41, representing pre-war conditions. A noticeable increase in births and decrease in deaths tending towards pre-war levels are evident, after making due allowance for non-receipt of returns for the respective years. It may be said in general that the study of vital statistics in the province has not revealed any abnormal feature which can be ascribed to food shortage. Periodical examination of destitutes and of vulnerable sections of the population carried out in the districts have also not revealed any signs of starvation among them.

Biometric studies :—The heights and weights of 480 elementary school children between 6 and 13 years (boys and girls) in Bellary, 957 in Anantapur and 241 in Cuddapah districts were compared with those of children in the city of Madras. The heights and weights of these mofussil children were found to be uniformly low compared to the Madras city standard for 1938.

Bombay.

General measures and work on nutrition :—Schemes for the improvement of the supply of milk in some of the towns have now been undertaken and are in progress. The supply of cheap subsidised milk for vulnerable groups is in progress in the City of Bombay and has been extended to some of the city suburbs. Nearly 2,550 maunds of milk are being distributed through 525 distributing centres in the city and 146 in the suburbs. In the city, 3,04,000 people as well as 42,000 in the suburbs are taking advantage of this scheme.

The Government are also considering opening up shops as a trial for toned milk to public at cheap rates. The milk is prepared at Anand by adding to fresh buffalo milk approximately 50% of reconstituted milk from separated milk powder and pasteurised. It is sold as a substitute for cow's milk containing 4% of fats and 9% solids not fat. This milk is sold at a cheaper rate than the prevalent prices. The subsidised milk distribution scheme had also been introduced in Poona during the year. Over 12,000 persons have been receiving this milk from 96 centres.

Shark liver oil :—During the year 4,000 gallons of shark-liver oil with high Vitamin A content have been produced. The collection of this oil is the result of training local fishermen in its production with the intention of developing it as a cottage industry along the coastline.

" Grow more food " campaign :—Work in connection with the cereals and " Grow more food " campaign has proceeded apace and efforts have been made to establish fruit tree nurseries. Vegetable extension schemes originally started for the military are still being continued and special efforts are being made for the distribution and sale of potatoes at cheap rates. A stock of dehydrated potatoes and dehydrated cabbages has also been put on sale in the City.

Community feeding :—The community feeding schemes in the Thana Police Station and among the Secretariat staff have been continued and

are gaining in popularity. The position of canteens in industries during the year has improved.

School feeding schemes:—School feeding schemes which were in progress have continued but it has not been possible to encourage the starting of others due to the scarcity of rationed articles.

Institutional feeding:—Lady dietitians have visited 120 institutions up to now and have studied their diets and other conditions pertaining to nutrition. Each institution has been advised on the modifications of diet which are necessary.

Feeding trials with yeast and with groundnut on the inmates of the David Sassoon Industrial School are progressing satisfactorily and regular observations are being made fortnightly. This experiment started on the 1st July, 1946.

Clinical nutrition surveys: Rapid clinical nutrition surveys of school children have been continued. Since January of this year 14,000 have been examined including 10,000 from primary schools in Bombay City. A report is being compiled.

Propaganda work:—Propaganda work is being done by lectures and exhibitions and by the issue of pamphlets in the main local languages, viz., Hindi, Gujerati and Marathi and the response has been very satisfactory.

The year under review was a year of declared scarcity in certain regions of the province. The main observation was that the scarcity mostly affected the non-agricultural population, as the agriculturists apparently had a certain amount of reserve, at least in some of the districts.

Eight million vitamin A and B (complex) tablets manufactured by the Haffkine Institute have been despatched every month to certain districts. These have been issued free of charge to children up to 10 years of age and to expectant and nursing mothers. Twenty million multi-vitamin tablets received from the Government of India, four million from the Haffkine Institute and two million received from other sources will be distributed in certain districts also.

(4) To consider the nutritive value of food yeast and a note by Dr. Bashir Ahmad on the utilisation of distillery and brewery yeasts.

The Committee consider that food yeast has now come to be accepted as a valuable supplementary food to the Indian diets. The results of work which is now in progress is being watched with great interest. They are of the opinion that brewery yeast which requires processing before use is suitable for medicinal purposes and recommend its use for the same. So far as distillery yeast is concerned, the Committee were of opinion that its purification was impracticable due to the presence of a large number of impurities.

(5) To consider the report on the suitability of "Maltavena" as a milk substitute in infant and child nutrition and the report on feeding trials.

The Committee studied with great interest the report published by Chick and Slack on the nutritive value of "Maltavena." They recommended that Professor V. Subrahmanyan of the Indian Institute of Science, Bangalore, should

be requested to plan and carry out investigations on the suitability of "Maltavena" in India. Professor V. Subrahmanyan has kindly consented to do this in conjunction with his soyabean work.

(6) Vegetable milks.

The following notes were presented by Professor V. Subrahmanyan :—

(i) "Digestibility of soya milk" by N. Krishnaswamy, S. S. De and V. Subrahmanyan.

(ii) "Nutritive value of groundnut milk" by H. S. R. Desikachar, S. S. De and V. Subrahmanyan.

(iii) "Effects of supplements on the biological value of the protein in ground-nut milk" by H. S. R. Desikachar, S. S. De and V. Subrahmanyan.

(iv) "A practical approach to the milk problem in India" (Vegetable milks—their nutritive value and ultimate practical possibilities) by S. S. De, H. S. R. Desikachar and V. Subrahmanyan.

(7) To consider the suitability of groundnut cake flour as a supplementary food.

The Committee considered the question referred to them by the Nutrition Committee of the Government of Bombay regarding the suitability of groundnut cake flour as human food. This question arose from an article in the 'Bombay Chronicle' dated the 20th September, 1946, which stated that this flour is not fit for human consumption. Dr. Subrahmanyan, who was quoted in the 'Bombay Chronicle' as the authority for this view, explained that he had been wrongly reported. He had never suggested that the use of groundnut cake flour was deleterious. His experiments had only shown that the supplementary value of groundnut cake flour when compared with cotton seed cake, sesame cake and cocoanut cake flour as a supplement to the poor Indian diet based on rice was low. Its value, however, as a supplement to diets based on other cereals such as wheat is established.

The Committee expressed the opinion that the fullest possible use of groundnut cake flour should be made provided it is prepared under controlled conditions and every care is taken to prevent rancidity.

REPORT OF THE MEETING OF THE LEPROSY ADVISORY COMMITTEE HELD ON THE 20th JULY, 1946.

The following were present:

MEMBERS.

Dr. C. G. Pandit. (*Chairman*).
Dr. V. R. Khanolkar.
Lieut.-Colonel G. Verghese.
Dr. Dharmendra. (*Secretary*).

VISITORS.

Major H. Lehmann.
Dr. S. R. Pandit.
Major T. C. Puri.

Lt.-Colonel H. W. Mulligan, Dr. J. Lowe, and Dr. R. G. Cochrane could not attend as they were out of India.

1. The Chairman informed the Committee of the formation by the Scientific Advisory Board of a committee to review the work on leprosy done under the Indian Research Fund Association as a basis for discussion regarding new lines of work on leprosy in India.

2. The Committee heard an account of the work by Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

Dr. Dharmendra explained that the therapeutic studies were grouped under three heads :—

(a) a trial of remedies reported upon favourably in the treatment of the disease;

(b) preliminary studies in rat leprosy of drugs likely to be of value in the treatment of human leprosy; and

(c) preliminary investigation in the preparation and testing of certain other drugs and antibiotics likely to be of value in the treatment of leprosy.

Under items (a) and (b) above drugs such as promin, diasone, and stilbamidine were under trial and under item (c) such drugs as hydnocarpic derivatives of sulphonamides a glucoside from the plant Hydrocotyle Asiatica and two antibiotics Aspergillin and Streptomycin were under investigation.

Dr. Dharmendra explained that most of these drugs except promin were not readily available and had to be prepared in the laboratory and in view of the favourable reports with their use in infections with certain acid fast organisms their trials in leprosy were indicated. Dr. Dharmendra also stated that in his experience penicillin was not effective in the treatment of leprosy.

3. The Committee considered an extract from the minutes of the fourth meeting of the Medical Research Liaison Committee for India, regarding the request for assistance of a pathologist in the study of the histo-pathology of leprosy by Dr. R. G. Cochrane and recorded the following :—

" While appreciating the willingness of the Medical Research Council to appoint and pay a pathologist from the United Kingdom for about a year

for this purpose, the Committee feel that a better approach to the problem might be to appoint a pathologist from India for such histo-pathological work, and if necessary to provide him with the facilities for training abroad with a view to equipping him with the necessary technical and theoretical knowledge for undertaking that investigation and thus utilising his services for a *long term study* of the problem in India.

They agree with the views expressed by Sir Henry Dale that such a person would be required to devote a number of years to this work, and that long continued detailed investigation would be required for this purpose. At the same time the proposal to send a histo-pathologist from the United Kingdom for about a year should be further investigated by the Secretary, Indian Research Fund Association".

REPORT OF THE LEPROSY ADVISORY COMMITTEE HELD AT 10-00 A.M. AT THE HAFFKINE INSTITUTE, BOMBAY, ON THE 12th DECEMBER, 1946.

MEMBERS PRESENT.

Dr. C. G. Pandit. (*Chairman*).
Lieut.-Colonel A. N. Chopra.
Dr. R. G. Cochrane.
Dr. V. R. Khanolkar.
Lieut.-Colonel H. W. Mulligan.
Dr. Dharmendra. (*Secretary*).

Colonel E. Cotter, I.M.S., Secretary, Scientific Advisory Board of the Indian Research Fund Association kindly attended the meeting.

IN ATTENDANCE.

Dr. Desai.
Dr. Figuredo.
Dr. P. V. Gharpure.
Lieut.-Colonel C. L. Pasricha.
Dr. R. Row.

1. The Chairman outlined briefly the work done by the Committee appointed to review leprosy work in India.

2. Dr. Dharmendra gave an account of the work done at Calcutta by the leprosy enquiry unit. The work included therapeutic, bacteriological, clinical and immunological studies. The last year's work and proposals for next year were discussed and it was recommended that work for the next year should be carried on the following lines:—

(i) Investigation of the therapeutic value in leprosy of promin and diasone;
(ii) Bacteriological studies;
(iii) Study of the eye lesions in leprosy; and
(iv) The long term study of selected cases of leprosy.

3. The proposal for a new enquiry under Dr. Cochrane for the study of histopathology of leprosy was considered and accepted. Since difficulty may be experienced in obtaining a high class pathologist it was suggested that the enquiry may start with reviewing the work already done on the cellular reaction in leprosy.

4. Section 2 of the minutes of the Medical Research Liaison Committee for India held on the 10th September, 1946, at London was considered. The Committee was grateful to the Medical Research Liaison Committee for the offer of help in obtaining therapeutic substances for trials in leprosy in India.

5. At the request of the Chairman, Dr. Cochrane gave his impressions about his recent visits to the United States and South America (See Appendix).

6. A reference made from the Clinical Research Advisory Committee of the I.R.F.A. was considered and it was agreed that in India priority should be given to leprosy for experimental treatment with streptomycin.

7. Colonel Cotter informed the Committee that the Governing Body of the B.E.L.R.A. have under consideration a proposal to send Dr. Dharmendra abroad for about eight months to contact leprosy workers and to see work in other countries. Dr. Dharmendra's itinerary will include a stay of three months in the United Kingdom to make a study of the histopathology of leprosy, and the remaining period will be spent in the United States, South America, Africa and Phillipines. The Committee agreed that Dr. Dharmendra's tour was very desirable but suggested that it would be advisable to study in England towards the end of the period of deputation, so that the time to be devoted to the study in histopathology would be extended with ease if needed.

APPENDIX.

The attention of the medical profession has been drawn to claims with regard to drugs of the sulphone group—Promin, Diasone, etc., in the treatment of leprosy. The active principle of these drugs is the toxic diamino diphenyl sulphone. The following reasons are given for the need of great caution in coming to any conclusions regarding their efficacy or the advocacy of these remedies for widespread use in the treatment of leprosy :—

(i) Adequate clinical trials have not yet been completed.

(ii) The published results do not indicate that even with the use of these drugs results can be expected under 18 months to 2 years. Further, the number of negative cases are still very few and a long enough period has not elapsed to know whether the disease will relapse or not.

(iii) The cost of treatment at present is prohibitive.

(iv) The drug is known to be toxic and therefore should not be administered apart from laboratory facilities so that adequate blood-level readings can be done as a check to dosage.

In this connection mention might be made that further research is needed to discover the best method whereby a high concentration of the drug may be produced in the skin for it is believed that the skin is the seat of greatest activity in leprosy and may be the key to the problem of therapeutics in leprosy.

Another matter which is receiving increasing importance is that of classification and the soundest classification appears to be that which attempts to correlate the immunologic and histologic findings with the clinical picture. It is held that while the S. American classification in the light of our Indian experience may need modifying, it is to date the most reasonable classification that has yet been put forward. The following is a brief summary of this classification as modified :—

I. Non-specific or uncharacteristic lesions :—

(a) Neural macule
(b) Prelepromatous macule

II. Tuberculoid leprosy :—

(a) Minor tuberculoid.
(b) Major tuberculoid.

This represents one of the extreme, or polar forms in the S. American classification.

III. Lepromatous leprosy.

This is the other extreme or polar form in the S. American classification.

IV. Intermediate, atypical or border-line cases. This is the no-man's land between leproma and tuberculoid and includes the sarcoidal forms.

A place has to be found for the neural anaesthetic type and also for the residual lesions. It may be necessary to place them in a separate category. The histologic picture of neural anaesthetic leprosy has not yet been worked out.

During the last two years research in leprosy has received a fresh stimulus and it is hoped that a more adequate study of the pathological processes will lead to a better understanding of the disease and more effective therapeutics.

REPORT OF THE MEETING OF THE PLAGUE ADVISORY COMMITTEE HELD IN NEW DELHI ON THE 20th JULY, 1946.

MEMBERS PRESENT.

Dr. V. R. Khanolkar. (*Chairman*).
Dr. P. V. George.
Dr. A. N. Goyle.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Dr. M. Sharif.
Dr. P. M. Wagle.
Major J. R. Dogra. (*Secretary*).

IN ATTENDANCE.

Dr. B. B. Dikshit.

The Committee considered Section 9(1) of the minutes of the first meeting of the Medical Research Liaison Committee for India held at the India Office, London, on the 15th November, 1945. They noted that laboratory work on several insecticides recommended for trial by the Medical Research Liaison Committee was in progress in the Haffkine Institute, Bombay, and field work to assess the value of DDT in the control of plague was under way in certain selected areas in the Madras province. The Committee noted that in this assessment cyanogas fumigation used as a routine preventive measure would serve as control. The Committee recommends that with the availability of other insecticides and rat poisons the field work would be profitably extended on the lines adopted for the DDT.

2. The Committee examined Section 7 of the minutes of the second meeting of the Medical Research Liaison Committee for India held at the India Office, London, on the 16th January, 1946, regarding an up-to-date documentation of the studies made in India on plague vaccine and on the chemotherapy of plague, and the letter from Lieut.-Col. Sir Sahib Singh Sokhey, I.M.S. to the Secretary, Indian Research Fund Association, New Delhi, in that connection. It was understood that his review would comprise of adequate information on the present position of the plague vaccine and the chemotherapy of plague.

3. The Committee considered and approved the progress report of the plague enquiry at the Haffkine Institute, Bombay.

4. The Committee considered a suggestion from Dr. Wagle regarding the necessity of establishing a dosage of plague vaccine on sounder experimental basis by initiating an investigation with the use of human vaccine sera on the lines of passive immunity experiments. Dr. Wagle was accordingly requested to proceed with his scheme of research. Dr. George agreed to assist in this investigation by supplying representative human sera whenever available.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met on the 11th December, 1946, the following members being present.

Dr. V. R. Khanolkar. (*Chairman*)
Dr. P. V. George.
Dr. A. N. Goyle.
Dr. C. G. Pandit.
Dr. M. Sharif.
Dr. K. V. Venkatraman.
Dr. P. M. Wagle.
Lieut.-Colonel Sir Sahib Singh Sokhey. (*Secretary*).

Lieut.-General R. Hay, Chairman of the Scientific Advisory Board of the Indian Research Fund Association very kindly attended the meeting.

The following were also present:—

Dr. R. Adiseshan.
Dr. B. B. Dikshit.
Major B. N. Khan.
Dr. K. V. Krishnan.
Dr. B. Sanjiva Rao.
Dr. D. W. Soman.
Mr. P. Bruce White.

1. *Plague enquiry under the Director, Haffkine Institute, Bombay.*

(a) *Plague vaccine :*—Lieut.-Colonel Sir Sahib Singh Sokhey presented data to show that the direct plague vaccine grown in casein hydrolysate medium for 14 days at 28° C., detoxicated and killed with 0·1 per cent formalin and preserved with phenyl mercuric nitrate, 1·5 mg., per 100 c.c., gave a mouse protective dose of 0·004 c.c. This vaccine has thus shown to be more potent than the original vaccine produced at the Institute in a meat-digest medium. During the year, the Institute produced a casein hydrolysate for parenteral administration and a direct plague vaccine was grown in this medium for comparison with the standard vaccine. This vaccine, grown in casein hydrolysate for parenteral administration, produced a poor vaccine with a mouse protective dose of 0·009 c.c.

Next, he presented some experimental data bearing on the keeping qualities of the direct casein hydrolysate plague vaccine as compared with the agar-grown vaccine. Both the vaccine retained their potency practically unaltered for a period of 20 months when stored at a temperature of 0°-4° C. But when stored at 37° C. the agar vaccine lost about half its potency after 2 months' storage, while the direct casein hydrolysate plague vaccine retained its potency practically unaltered up to 14 months' storage. After that period, both the vaccines showed a great deterioration. Colonel Sokhey suggested that these experiments should be repeated to confirm the results.

During the course of the discussion, Dr. K. V. Krishnan said that the observation that the casein hydrolysate plague vaccine gave a greater protec-

tion than the agar vaccine, was an interesting one and was in keeping with recent observations on the immunogenic response for different types of vaccines. It had been observed by some workers that vaccines of certain organisms are administered along with protein-split products of a non-specific nature. They induce better immunity response. He wanted to know whether similar studies had been made on the causes of the improved quality of the casein hydrolysate plague vaccine. Was it due (i) to the presence of hydrolysed proteins in the vaccine in the form of amino-acids and peptids, or (ii) to any alteration in the organism itself due to the quality of the medium in which it is grown ? Studies to elucidate these points would be extremely important not only for plague but for other vaccines.

Colonel Sokhey welcomed Dr. Krishnan's remarks and said that studies to fractionate out by newer methods of precipitation of protein fractions with ethyl alcohol at low temperature were being planned and as soon as arrangements were completed, an attempt will be made to separate out different fractions that were immunogenic in the casein hydrolysate plague vaccine. In this connection Colonel Sokhey told that Dr. Karl Meyer, the head of the George Hooper Foundation, had already reported some work on these lines. The effect of casein hydrolysate itself in conjunction with agar-grown suspensions will also be tried.

(b) *The effect of DDT dust on the breeding of the Indian rat-fleas, Xenopsylla cheopis* and *X. astia* : Dr. M. Sharif presented experimental evidence to show that the residual effect of DDT on fleas is very long and it was found effective up to 396 days in jar experiments and 428 days in burrow experiments (the periods tried so far). There was no deterioration noticed due to volatilization in the rat burrows. Unequal distribution of DDT in burrows, however, gave inconsistent results. The development of cracks also disturbed the distribution of DDT in artificial rat burrows when used over a long period of time. DDT was found not to be effective against eggs, and pupae well protected with in cocoons.

Dr. P. V. George wanted to know whether the jars and burrows were kept open during the period of these experiments. Dr. Sharif replied in the affirmative.

(c) *Standardization of the dosage of plague vaccine* :—Dr. P. M. Wagle described the work that he had recently started to explore the possibility of standardization of the dosage of plague vaccine. He found that 2·5 c.c. of serum of a patient convalescing from plague would protect a mouse against 10 m.l.d. of plague organisms, while 5 c.c. of normal human serum gave no protection. He intended to employ passive immunity for measuring the adequate dose of plague vaccine for protection in man.

Dr. K. V. Krishnan remarked that standardization of plague vaccine with a view to ascertaining the optimum prophylactic dose to be administered to human beings was a subject of great importance and any inquiry on that subject deserved whole-hearted support. But the methods to be adopted for assessment of the optimal dose deserved very careful consideration. All factors concerned in the mechanism of immunity in plague were not fully understood, and to base the dose purely on a study of the antibody produced may not be wholly acceptable. Furthermore, there may be marked variations in different individuals.

Dr. Dikshit observed that he agreed with Dr. Wagle regarding the methods he had adopted for standardization, but pointed out that as regards observations on the measurement of immunity in convalescents from plague, care should be taken to take serum from patients who had not been treated with sulphonamides. He was of the opinion that under the influence of sulphonamides, the full mechanism of human immunity against plague infection may not be brought into operation and, therefore, the standard of immunization set may not be adequate.

Dr. K. V. Venkatraman welcomed this fresh approach to the problem of standardization of the dose of plague vaccine. The investigation of the level of demonstrable serum antibodies during convalescence after an attack of plague and after inoculation with vaccine will be of great interest, but it would be necessary to bear in mind that the level of serum antibodies is only a part of the picture in the immunity processes. The readiness with which a certain predetermined level can be attained by a further stimulus may also be considered.

The committee approved of the continuance of these enquiries during the year 1946-47 and recommended that the grants asked for may be sanctioned.

2. *Plague enquiry in the Nilgiris under the Director of Public Health, Madras.*

Dr. P. V. George gave the salient features of the work being done on cyanogas fumigation and DDT. He drew the attention of the committee to the entirely different climatic and faunal conditions of Nilgiris as compared with the Husur Taluk, where similar work had been carried out previously and stressed the importance of the work under these different conditions. He regretted that the inquiry failed to find adequate accommodation at the Pasteur Institute, Coonoor, and which had considerably hampered his work. He told the committee that he was requesting the I.R.F.A. to permit him to use Rs. 3,000/- from the unexpended portion of the grant for the current year for building two rooms at the Pasteur Institute, Coonoor, for housing the experimental animals and other work in connection with this inquiry. The committee was unable to express any opinion on this issue, as the committee had no papers before them and suggested that Dr. George should approach the Secretary, I.R.F.A., in this connection.

Major B. N. Khan wanted to know whether the live fleas which Dr. George had found from one of the burrows which had been sprayed with DDT were found in the main burrow or from a tributary springing from the main burrow.

Colonel Sir Sahib Singh Sokhey pointed out that perhaps Major Khan's question arises from his desire to know whether the kerosene oil solution of DDT sprayed with a stirrup pump was uniformly distributed in the burrow. He pointed out that if DDT is applied with the kerosene oil solution, it is very unlikely to be distributed evenly. He suggested that in such experiments a finely divided powder of DDT would be more effective and suggested to Dr. George that in his future work, he should use DDT powder for applying to rat burrows.

Dr. Sharif observed that in order to see the effect of DDT on the flea population under natural conditions, the use of 10 per cent DDT dust for killing fleas in the burrows and of 5 per cent spray of water dispersible powder in solution in water for killing fleas in exposed spaces such as floorings, etc., is suggested. For this purpose, two small villages showing similar flea indices

should be selected, one treated with DDT and the other to serve as a control. Their flea indices should be compared after stated intervals. DDT had been found very effective in controlling a plague epidemic in 1945 at Tumbes in Peru. A similar trial in an Indian village was suggested.

The committee recommended the continuation of the enquiry.

3. *Plague enquiry in Hosur taluk, Salem district, under the Director of Public Health, Madras.*

Regarding the plague enquiry conducted in Hosur taluk, salem district, between 1941-46, the committee requested Dr. George to write up a comprehensive review of the work and present it to the committee at their next meeting.

4. *Consideration of copy of Section 3 (a) (v) of the minutes of the fifth meeting of the Medical Research Liaison Committee held at the India Office on the 10th September, 1946, together with certain documentation.*

Of the valuable suggestions received, special attention was drawn to Professor Buxton's suggestion that more effective distribution of DDT and gammexane in burrows might be obtained by the use of smokes. In view of the difficulties experienced in obtaining a uniform application of these insecticides in rat burrows the workers were requested to experiment with smokes. Their attention was drawn to the ready-made generators available from Fumite Distributors, Ltd., and I.C.I. Workers were asked to write to the Secretary, I.R.F.A., for their requirements of these generators.

REPORT OF THE THIRD MEETING OF THE CLINICAL RESEARCH ADVISORY COMMITTEE HELD IN NEW DELHI ON THE 22ND JULY, 1946.

Members present :—

Dr. A. N. Goyle. (*Chairman*).
Major A. T. Andreasen, I.M.S.
Dr. S. W. Hardikar.
Lieut.-Colonel Jaswant Singh, I.M.S.
Dr. V. R. Khanolkar. (*Secretary*).

Invitees :—

Major P. D. Bhave.
Dr. B. K. Pal Choudhury.
Dr. R. E. Dadachanji.
Dr. M. B. Daver.
Major J. R. Dogra, I.M.S.
Lieut.-Colonel S. D. S. Greval, I.M.S.
Major H. Lehmann.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Dr. V. N. Patwardhan.
Dr. M. V. Radhakrishna Rao.
Lieut.-Colonel B. K. Sheorey, I.M.S.

In the absence of Dr. Kini, Dr. A. N. Goyle took the Chair.

1. The minutes of the 2nd meeting of the Clinical Research Advisory Committee held in New Delhi on the 29th November, 1945 were adopted.

2. The Committee considered the suggestion from the Nutrition Advisory Committee regarding the formation of a scheme for the study of lathyrism in Central Provinces & Berar. A small Sub-Committee was appointed to draft tentative proposals for this purpose :—

Major A. T. Andreasen.
Major P. D. Bhave.
Dr. S. W. Hardikar.
Dr. V. R. Khanolkar.
Dr. V. N. Patwardhan.

The report of the Sub-Committee is attached as Appendix.

3. The Committee realised that it was too premature to consider progress reports of enquiries on clinical research sanctioned by the I.R.F.A. in 1946 as many of them had only recently started. They, however, were glad to hear an account of investigations on Naga sore from Dr. S. R. Pandit. During the course of discussion, Dr. Radhakrishna Rao expressed his opinion regarding the role of nutritional deficiency and the improvement after the administration of vitamin A concentrates. Dr. S. R. Pandit, however, reported that careful surveys of populations of tea plantations in Assam did not afford any evidence in support of nutritional deficiency playing a part in the etiology of Naga sore. This was also supported by the observations of Major Andreasen on the therapy of *Ulcus tropicum*.

4. *Chemotherapy of tetanus :—*

The Committee considered Section 9(3) of the minutes of the 1st meeting of the Medical Research Liaison Committee held on the 15th November, 1945 at the India Office, London. During the course of discussion it was pointed out that under the existing conditions in most of the Presidency and Civil Hospitals it would be difficult to undertake therapeutic trials on V-187, particularly as these cases occur sporadically and do not come early under the observation of the physician or surgeon concerned. It was suggested by Major Andreasen and supported by other members that a scheme for the segregation and organised treatment of such cases may be submitted to the Secretary, I.R.F.A. for circulation to obtain the co-operation of the big hospitals in the country. On receipt of further information from these institutions a request would be made to the Medical Research Council for a supply of the drug.

5. *Consideration of section 2 of the minutes of the 3rd meeting of the Medical Research Liaison Committee held on the 18th March, 1946, at the India Office, London :—*

The Committee were grateful to the Medical Research Liaison Committee for the interest taken by them and would like to request the Secretary, I.R.F.A., to communicate to Professor Himsworth their appreciation of the trouble taken by him in preparing a memorandum as a basis of work on the relationship of diet to liver disease.

(a) *Infections of the feet :*—Dr. Khanolkar stated that his attention had been drawn to the recent American work on the treatment of fungoid infections of the feet. He presented an abstract of some of this work and stated that he would be glad to send a bibliography of the recent work to any member of the Committee who may be interested in these infections. Many of those present showed interest and requested that further information be supplied to them.

(b) *Infantile biliary cirrhosis and related experimental studies :*—The Secretary informed the Committee that a joint programme for enquiry on this problem had been drawn up in February, 1946 by different workers referred to in the note to para (viii) on page 3 of the report of the 2nd meeting of the Clinical Research Advisory Committee. They now had also the benefit of suggestions from Sir Edward Mellanby and Professor C. R. Harrington as well as the very useful memorandum by Professor Himsworth. The Secretary was glad to inform the Committee that Major Lehmann was able to attend the meeting and that before going to Uganda he might be of assistance in co-ordinating work on similar lines between workers in Africa and India.

During the course of discussion various aspects of the problem were presented. The experimental work at the Haffkine Institute, Bombay, was referred to by Dr. Radhakrishna Rao. Major Lehmann referred to the work of Trowell and Holmes in Uganda and to that of the Indian workers he had seen in this country. He also referred to the work of Dr. Krishna Rao of Bangalore and described the clinical trials by the Mysore team of workers now in progress.

Major Andreasen referred to his experiments with intestinal strangulation in dogs and the resulting changes in livers and kidneys. He also referred to his clinical observations on hepatic insufficiency in patients with sub-clinical colitis which is extremely common in this country and suggested that a conditioned malnutrition thus set up might be a major mechanism in causing liver fibrosis.

6. *Medical research organisation in the army in India :—*

Major Lehmann pointed out that a permanent medical research organisation has been set up by the Medical Directorate, G.H.Q.(I), which would be happy to co-operate closely with the civil research organisations in India. The main lines of research will be on typhus, nutrition, anaemia and protozoology. The Committee expressed their appreciation of the offer of co-operation and it was suggested that the I.R.F.A. be requested to co-opt the Assistant Director of Pathology, G.H.Q.(I), as a member of the Clinical Research Advisory Committee.

7. The following three topics were referred to :—

(a) Lieut-Colonel S. D. S. Greval : The tellural influences in India on the diseases of temperate climate.

(b) Lieut.-Colonel Jaswant Singh : The need for collection of clinical information regarding the use of Paludrine in cases of malaria.

(c) Major Andreasen : The necessity for better appreciation of the climatic conditions in the tropics in relation to the standard treatment of shock.

APPENDIX.

SCHEME FOR INVESTIGATION OF LATHYRISM.

The Sub-Committee, mentioned in para 2, met and drew up the following scheme which they recommend be put forward by the I.R.F.A. :—

1. The base of brain, spinal cord, and a 2" segment of the sciatic nerve from each side, be taken as soon as possible after death from every available body of persons who have suffered from lathyrism.

(Technique of removal of these parts—Appendix A.)
(Technique of preservation of the specimens—Appendix B.)
(Instructions for sending the material to the pathologist—Appendix C.)

2. All cases suspected of the disease should be notified to the civil surgeon of the district, who will in turn inform the Director of Public Health, Central Provinces and Berar.

In this way, the location of new cases and of groups of cases will be made known to the field workers.

3. Any person admitted to district or sub-divisional hospitals or Dispensaries, who gives a history of having suffered from lathyrism and dies in hospital should have an autopsy performed as early as possible and the material mentioned in para (1) collected and preserved and dispatched for study.

4. It is proposed that a suitable person be sent to the laboratories of the Tata Memorial Hospital, Bombay and the Neuropathology Department of the Central Military Pathology Laboratory, Poona, for training in the technique of histopathology of the Central Nervous System.

5. It is recommended that this worker, after training, be attached to the Clinical Research Unit, Tata Memorial Hospital, Bombay. He would undertake the preparation and examination of the collected human material, and such animal material, experimental and otherwise, as might be sent by workers under grant from the I.R.F.A. or from other approved investigators.

6. The Committee recommends that a rapidly mobile unit with a clinician and a biochemist would be the most profitable method of collecting the clinical and biochemical data for investigation of this problem.

7. It is recommended that an effort be made to secure an army mobile laboratory fitted for clinical pathology to realise the mobile unit suggested in para (6).

8. It is strongly recommended that a clinician and a biochemist *interested* in this work should be deputed from the Central Provinces Service, if available, or from such other sources as may be deemed suitable.

9. The Committee would like to recommend that the Director, Imperial Veterinary Research Institute, be approached for co-operation by undertaking an investigation into the relative potentiality of *Vicia sativa* and *Lathyrus Sativus* in producing lathyrism in horses.

10. The Committee recommended the following broad plan of field investigation :—

A. *Clinical.*

1. Detailed clinical history and examination. (Appendix D.)

2. Special dietary history.
3. Follow up according to above detailed plans.

AIM: To discover the earliest signs and symptoms and dietary associations.

B. *Biochemical:—*

1. Set group of blood, urine and C.S.F. examinations.
2. Follow up examinations. (Appendix E.)

Clarification is required on the following points:—

1. Age groups affected.
2. Earliest symptoms and signs.
3. Accurate identification of the vetches eaten.
4. Variations in quantity of vetches eaten.
5. Evidence for seasonal and germinal variations in toxicity of vetches eaten.
   (a) Identification of the toxic factor of the vetch.
   (b) Study of its action.
6. Epidemicity.
7. A suitable experimental animal.
8. An investigation of the variations in symptoms reported in experimental animals.
9. Investigation of pathological material from these animals.
10. Precipitating conditions in man.
11. The exact nature of the lesion in man:—

    Is the lesion primarily nervous, or are these signs secondary to a vascular lesion or a nutritional deficiency?

The work of: Acton (I.M.G. 1922. 57. 141)
Acton & Chopra (I.M.G. 1922. 57. 412)
Anderson et al. (I.J.M.R. 1925. 12. 613)
McCombie Young (I.J.M.R. 1927. 15. 453)
Stockman (J. Pharm., Exp. Therap. 1929. 37. 43.)
Stockman (J. Hyg. 1931. 31. 550)
Stockman (J. Hyg. 1934. 34. 145)

should be consulted.

(Appendices to follow as prepared by allocated members of the Sub-Committee).

REPORT OF THE FOURTH MEETING OF THE CLINICAL RESEARCH ADVISORY COMMITTEE HELD IN THE HAFFKINE INSTITUTE, BOMBAY, ON THE 10th DECEMBER, 1946.

MEMBERS PRESENT.

Major A. T. Andreasen. *(Chairman).*
Dr. R. N. Cooper.
Dr. A. N. Goyle.
Dr. S. W. Hardikar.
Lieut.-Colonel Jaswant Singh.
Dr. Jivraj N. Mehta.
Dr. J. C. Patel.
Colonel A. Sachs.
Dr. M. J. Shah.
Captain E. J. Somerset.
Dr. B. B. Yodh.
Dr. V. R. Khanolkar. *(Secretary).*

Lieut.-General R. Hay, I.M.S., Chairman of the Scientific Advisory Board of the Indian Research Fund Association kindly attended the meeting.

Dr. M. N. De, Rao Bahadur Captain M. G. Kini, Dr. P. Kutumbiah and Major F. A. B. Sheppard were unable to attend.

BY INVITATION.

Dr. R. Aidseshan.
Dr. R. G. Chitre.
Colonel Sir Ram Nath Chopra.
Dr. A. Das.
Mr. N. C. Datta.
Dr. B. B. Dikshit.
Colonel G. K. Fulton.
Dr. P. V. Gharpure.
Dr. K. V. Krishnan.
Dr. M. N. Lahiri.
Major H. Lehmann.
Colonel F. M. Lipscomb.
Dr. T. Bhaskara Menon.
Dr. V. R. Naidu.
Dr. C. G. Pandit.
Lieut.-Colonel C. L. Pasricha.
Dr. V. N. Patwardhan.
Major T. C. Puri.
Dr. M. V. Radhakrishna Rao.
Dr. D. L. Shrivastava.
Dr. D. W. Soman.
Major B. L. Taneja.
Major O. P. Verma.

The Chairman before taking up the items on the agenda welcomed the new members of the Committee, Colonel Sachs, Director of Pathology, G.H.Q.,

Dr. Jivraj Mehta, Drs. M. J. Shah, J. C. Patel, R. N. Cooper and B. B. Yodh. The new members had been included in order to establish a close correlation between researches under Army organisations and civilian research activities, and to represent the work in progress at the place where the meeting was held.

Item 1 :—The minutes of the third meeting of the Clinical Research Advisory Committee held in New Delhi on the 22nd July, 1946, were referred to by Dr. Khanolkar, Secretary of the Committee. He pointed out that Major Andreasen had kindly agreed to submit notes on "Suggested lines of work on Shock" and on "A scheme for the study of Chemotherapy in Tetanus." These notes are included as *Appendices I and II*. The minutes of the third meeting were then adopted.

Item 2 :—The Committee then proceeded to consider the progress of work on enquiries sanctioned during the year 1946-47.

(i) Work at the clinical research unit under Dr. V. R. Khanolkar on 'Vitamin studies in oral cancer; and experimental production of liver cancer in rats.' The Committee approved the work carried out by the unit and recommended its continuation.

The Committee then considered the reports on the following enquiries and recommended as follows :—

(ii) Enquiry on Naga sore under Dr. S. R. Pandit, Director, Pasteur Institute, Shillong.

Recommended for one year only.

(iii) Enquiry into hypoproteinaemia and other conditions associated with malnutrition under Dr. John Lowe at the School of Tropical Medicine, Calcutta.

Discontinued.

(iv) Enquiry into erythroblastosis foetalis and its association with the Rh factor under Lieut.-Colonel S. D. S. Greval, I.M.S., Imperial Serologist to the Government of India at the School of Tropical Medicine, Calcutta.

Recommended for consideration of the Scientific Advisory Board, if funds permit (low priority).

(v) Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

Recommended for one year.

Colonel Sachs pointed out the importance of this subject to the Army and stated on behalf of G.H.Q. that he welcomed a co-operation between his unit and the unit working on this subject at the School of Tropical Medicine, Calcutta.

(vi) Enquiry into the infections of the feet under Rao Bahadur Captain M. G. Kini at the Stanley Hospital, Madras.

Recommended for one year only.

(x) Dental Research Unit under Dr. C. D. Marshall Day at the de Montmorency College of Dentistry, Lahore.

It was recommended that the proposal be returned for resubmission and clarification within the limits of requirements set out by the I.R.F.A. for submitting proposals.

(xi) Enquiry on the relationship between the measurements of vertebrae and body size of Indians under Dr. R. G. Ginde at the Seth G. S. Medical College, Bombay.

Recommended for the next year.

(xii) Enquiry into causes of blindness under Captain E. J. Somerset, I.M.S., at the Medical College, Calcutta.

Recommended for one year with a review at the end of the year of work carried out since the start of the enquiry.

(xiii) Typhus enquiry under Dr. D. W. Soman at the Haffkine Institute, Bombay.

Recommended for one year.

During the course of discussion Col. Sachs reviewed the work on typhus carried out by the army group in countries adjacent to India and in this country. He stated that three problems presented themselves as a result of this work.

(i) Is there such disease as X_2 typhus?

(ii) The preparation of a satisfactory inoculum.

(iii) Further study of human pathology of the disease.

Colonel Sachs presented a note on the distribution of typhus fever in army in 1943-46 reviewing some of the work on typhus carried out by the army for guidance of civilians interested in the problem (*Appendix III*).

Before proceeding to a consideration of new enquiries the Committee noted a suggestion by Dr. Jivraj Mehta that as it was difficult for members of the Committee to get a clear idea of some of the proposals from the cyclostyled notes, the proposers may be advised to contact workers from their locality, who would be likely to attend the meetings of Advisory Committees, and to explain their problems to them, so as to enable them to sponsor their proposals. The Committee approved of the suggestion and resolved to forward it to the Secretary, I.R.F.A. with a recommendation for its incorporation in the general circular letter which is issued annually asking for proposals for new enquiries.

Item 3 :—The Committee then considered proposals for new enquiries :—

(ii) Enquiry on myopia in relation to the nutritional state of the body under Dr. G. S. Pendse, Poona.

After discussion it was decided that in view of the importance of obtaining basic data on a study of refraction in the population in India, specially children and particular racial groups, the proposer of this problem be asked to contact Captain Somerset and Dr. V. N. Patwardhan with a view to submit a fresh proposal at the next meeting.

The proposal as set out was not recommended.

(v) Enquiry on the incidence of Brucella infection in Bombay under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

Not recommended.

(vii) Enquiry on the effect of vitamins C and B in diabetes under Dr. S. Banerjee, Diabetes Research Worker, School of Tropical Medicine, Calcutta.

Recommended for one year only.

(viii) Enquiry into venereal diseases in Calcutta with a view to determine their intensity, latency and resolution under Lieut.-Colonel S. D. S. Greval, I.M.S., Imperial Serologist to the Government of India, Calcutta.

Not recommended.

(ix) Enquiry on tuberculosis under the Director, School of Tropical Medicine, Calcutta.

Not recommended.

(xi) Enquiry into the incidence and aetiology of parodontal diseases under Dr. K. L. Shourie at the Rochester University, New York, U.S.A.

Not recommended.

(xii) Survey of hookworm diseases in the Jharia Coalfield Settlement Area under Dr. A. K. Mukherji, Officer-in-Charge, Helminthological Department, School of Tropical Medicine, Calcutta.

Recommended for one year only.

(xiii) Enquiry into the causes of anaemias in the Assam valley areas under Major J. R. Kerr, Superintendent, Berry White Medical School, Dibrugarh.

Not recommended.

(xiv) Enquiry into typhus in Bengal at the All-India Institute of Hygiene and Public Health, Calcutta.

Recommended for one year only.

Item 4 :—The report of the Sub-Committee of research workers on cirrhosis of the liver was adopted and is appended as *Appendix IV*.

Item 5 :—The report of the Sub-Committee for studies on lathyrism was adopted and is appended as *Appendix V*.

Item 6 :—The Committee recorded the note from Colonel Pasricha, regarding the question of the Association encouraging research in tuberculosis.

Item 7 :—After some discussion it was decided that as this was a request for clinical test of a drug the Committee should have further information from the Public Health Commissioner with the Government of India on the policy adopted towards problems of this nature.

Item 8 :—A note on Section 3(d) of the minutes of the 5th meeting of the Medical Research Liaison Committee for India held on the 10th September, 1946 at the India Office, London, was read and noted.

Item 9 :—A note was read from the Director General, Indian Medical Service on the expected quota of Streptomycin. Since the quantity available would be extremely small and therefore cannot conveniently be used for a clinical trial in tuberculosis, the Committee recommended that the monthly supply be accumulated for a period of 6 months and that the question as to how the accumulated quantity should be used be reconsidered at the next meeting, subject to information concerning the keeping properties of the drug. It was further recommended that this matter be brought to the notice of the Leprosy Advisory Committee.

Item 10 :—Colonel Sachs drew attention of the Chairman to the manner in which papers were presented and pointed out that it was customary in America and in the army that any proposal for a grant-in-aid of a research work

is required to contain not only a full statement by the worker as is at present done here but should also contain a front page on which:

(i) *the object of the work* is clearly set out in one sentence;

(ii) *the conclusions* expected or already drawn in a series of short sentences (not exceeding three);

(iii) the exact *requirements* precisely set out; and

(iv) a blank space headed *recommendations by the committee*;

Item 11:—Professor S. W. Hardikar stressed the need for laying down some principles for the guidance of members which may enable them to evaluate proposals of research submitted for their consideration. The Committee felt that the matter was of great importance which should receive consideration by the I.R.F.A. A note by Professor Hardikar as elaborated by Dr. C. G. Pandit is incorporated in this report.

NOTE BY DR. S. W. HARDIKAR.

As a member of the Committee I have experienced considerable difficulty in arriving at a decision as to the acceptance or rejection of a scheme submitted for consideration, since the immediate policy of the I.R.F.A. was not sufficiently clear for the guidance of members. The general aims of the Association as laid down in its memorandum have necessarily to be wide. The difficulty was mainly in deciding which type of research should be considered as routine work which need not be financed by the I.R.F.A. My own feeling in this matter is that in the present state of research in this country a good part of the research work to be promoted might fall in the category of routine.

Workers permanently employed in institutions presumably for research have to come to the I.R.F.A. year after year for grants-in-aid. This is not in the best interests of research either for the worker himself or of the staff employed on such schemes on a yearly basis. I, therefore, feel that following the example of the Clinical Research Advisory Committee, which has laid down criteria by which schemes are to be judged, it would be advisable to appoint a sub-committee to lay down guiding principles regarding policy for promotion of research.

REMARKS BY DR. C. G. PANDIT ON DR. HARDIKAR'S NOTE.

The difficulties pointed out by Professor Hardikar were due to the fact that no funds were earmarked for research in most of the institutions maintained by Governments and other corporate organisations. It was therefore necessary for workers who were anxious to undertake investigations relating to their routine work or problems in which they were interested to seek the financial assistance of the I.R.F.A. The remedy, therefore, lay in convincing such organisations regarding the necessity for the allocation of funds for research in their own institutions, and the advantages which would accrue to them from such allocations. It is, therefore, suggested that the I.R.F.A. should assess the suitability of any proposals for grants-in-aid on the basis of the criteria laid down by the Clinical Research Advisory Committee at their 2nd meeting whether the proposals were submitted by individuals or by institutions through their heads. They should also ascertain whether the proposals submitted to them if originating from an institution could not have been carried out from its own resources.

APPENDIX I.

A note on suggested lines of work on shock

By

PROFESSOR A. T. ANDREASEN, F.R.C.S. (ENG.).

Experimental work and clinical observation have not definitely settled the mechanism and treatment of shock as is evident from a perusal of the current literature. It is, therefore, very essential that all concerned (especially teachers of medicine and surgery) should interest themselves in this interesting subject.

The object of the following brief note is to bring to the attention of clinicians and other interested workers, a number of problems requiring further work, and a number of modifications in treatment of shock requiring further extension of clinical study.

1. The authoritative statements on shock and resuscitation require urgent revision in view of recent work, since the average practice now diverges widely from the directions given in the manuals issued by Government authority.

2. Especial attention is required in the matter of climatic conditions and the modification they set upon the general recommendations and statements made by authority of Research Council.

3. Attention to clarification of statements regarding secondary shock by addition to the usual statement of its definition of the point that there is considerable evidence to show that this condition is characterised by vasoconstriction in the capillaries and is of metabolic nature; primary shock being localised in the arteriorles and characterised by vasodilation of nervous origin.

Evidence of present confusion of thought on these points can be had in the numbers of published accounts of the use of various types of nerve block and the use of vasoconstrictors in the treatment of cases suffering from secondary shock. If the true basis of the condition was realised it would be evident that Vaso-Constrictors are contra-indicated and that no form of nerve block can possibly inhibit secondary shock.

4. Question of the possibility of local accumulation of fluid in the injured parts being the killing factor requires revision in the light of the fact that the work of Phemister and Blalock failed to consider whether severe shock can be fatal even if sufficient plasma (to maintain blood volume) be given from the earliest stages.

Chess *et alia* (1) have shown this to be so.

5. Chambers *et alia* (2) (3) have confirmed that circulation of chemical bodies corresponding to the successive stages of shock takes place.

6. The presently accepted irreversibility of shock has been challenged very seriously, if not overthrown by the work of Chambers *et alia* (2) (3) and of Levine-Soskin (4) *et alia*.

7. The incorrectness of the theory of endless leakage of plasma through damaged capillaries and hence the necessity of colloids to keep the blood stream filled, has been shown by these same workers.

8. There is much evidence for the superiority of proper amounts of saline over plasma (2) (5) (6) (7) (8) (9) (10) and of saline—sodium bicarbonate solution.

9. Red-cell-saline suspensions have been shown superior to even heparinised plasma for haemorrhagic shock.

The teaching that plasma is a specific for shock is therefore unsound and can be shown experimentally to be dangerous.

10. The statements which combine "Saline or Saline-glucose"—together, require modification. A patient in shock requires that his metabolism be temporarily lowered as far as possible; dextrose is a metabolic stimulant. This substance, and aminoacids are not indicated until the crisis is over.

11. In 1937 proof was given of the inhibition of shock and necrosis by application of low temperatures. Still the instructions remain regarding warmth. Temple Fay (14) clearly demonstrated the value of reduced temperatures in treatment of shock in 1940-42. Other observations support these findings. (15) (16) (17) (18) (19) (20) (21) (22) (23) (24). This has nothing to do with the fact that "Exposure" (either to cold or heat, by loss of fluid, warming or shivering increase metabolism and so deplete body reserves) increases shock.

12. The radical application of low temperature locally controls shock and saves life to an even greater degree (23) (24) (25) (26) (27) (28) (29) (30).

13. Refrigeration has been shown to be the only agent which inhibits both primary and secondary shock; the only anaesthetic possible in very ill patients, and the only one which has never caused any deaths.

14. Tourniquet shock is now a well established fact. Yet the recommendation that a tourniquet should be released at short intervals still appears and is still being taught without qualification.

15. Application of the principle of a tourniquet before release of a crushed limb to arrest shock is nowhere mentioned.

16. Application of combined refrigeration and tourniquet principles in the prevention of haemorrhage, shock and infection are not evident.

(Some of these points can be found scattered throughout various official memoranda, but are not yet collected into the short official statement on wound shock and its treatement).

Shock, infection, gangrene, frostbite, immersion foot all respond well to refrigeration. Pain is cut out and metabolism lowered. The method is applicable to burns with equally good results.

Emphasis on these basic principles is of the greatest importance in the tropics where life can be so easily lost by mishandling of what was originally simple dehydration or easily reversible and early shock. Where much treatment is carried out by good sincere workers with the unfortunate disadvantage of a training in science and medicine where reverence of authority—necessary successful circumvention of examining bodies—has become imperceptibly and unintentionally substituted for training in powers of independent observation and reasoning.

Bibliography.

1. Chess, S., Chess, D., & Cole, W. H. Arch. Surg. **49** : 147-155, 1944.
2. Allen, F. M. Arch. Surg. **38** : 155-180, 1939.
3. Chambers, R., Zweifach, B. W., & Lowenstein, B. E. Proced. Soc. Exper. Biol. & Med. **56** : 127-129, 1944.
   Am. J. Physiol. **142** : 80-93, 1944.
   Chambers, R. & Clowes, G. H. A. Surg. Gynae & Obst. **80** : 593-608, 1945.
4. Levine, R., Huddleston, B., Persky, H. & Soskin, S., Am. J. Physiol. **141** : 209-215, 1944.
5. Allen, F. M., Am. J. Surg. **60** : 335-348, 1943.
   61 : 79-92, 1943.
   62 : 80-104, 1943.
6. Katz, L. N., Friedberg, L. & Asher, R. Am. J. Physiol. **140** : 65-71, 1943.
7. Scott, C. C., Worth, H. M. & Robbins, E. B. Arch. Surg. **48** : 315-318, 1944.
8. Rosenthal, S. M., Public Health Reports, **58** : 513-522 ;
   „ „ „ 1429-1436, 1943.
   „ „ „ **59** : 637-658 : 1944.
   „ „ „ **60** : 378-381, 1945.
   „ „ „ 401-418, 1945.
9. Fox, C. L., J.A.M.A., **124**, 207-212, 1944.
   Fox, C. L. & Keston, A. S., Surg. Gynae, & Obst. **80**, 561-567, 1945.
10. Underhill, F. P., Ann. Surg. **86** : 840-849, 1927.
11. Ivy, A. C., Greemgard, H., Steom., Grodings, F. S., and Dutton, D. F. Surg. Gynae. & Obst. **76** : 85-90, 1943.
12. McKee, F. W., Laycock, S. F., Martens, F. G., & Nicholl, R. J., Surg. Gynae. & Obst. **78** : 509-514, 1944.
13. Rhoads, J. E., Wolff, W. A., Daltonstall, H. & Lee, W. E., Clinics ; **1** : 37-42, 1942.
14. Fay, F., Pennsylvania M. J., **46** : 332, 1943.
    J.A.M.A., **121** : 1109, 1943.
15. Waters, R. M., J.A.M.A., **121** : 783, 1943.
16. Perry, I. H., J.A.M.A. **121** : 966, 1943.
17. Wright, R. D., & Devine, J., M.J. Australia **1** : 21-27, 1944.
18. Elman, R., Cox, W. M., Lischer, C., & Mueller, A. J., Proc. Soc. Exper. Biol. & Med. **51** : 350-351, 1942.
19. Cleghorn, R. A., Canad, M.A.J. **49** : 363-367, 1943.
20. Wakim, K. G., & Gatch, W. D., J.A.M.A., **121** : 903-907, 1943.
21. Antos, R. J., Proc. Soc. Exper. Biol. & Med. **56** : 60-63, 1944.
22. Green, H. D., & Bergeron, Surgery **17** : 404, 1945.
23. Ricca, R A., Fink, K. Katzin, L. I., & Warren, S. L., J. Clin. Investigation **24** : 127-139, 1945.
24. Katzin, L. I., Ricca, R. A., & Warren, S. L., J. Clin. Investigation **24** : 149-151, 1945.
25. Allen, F. M., Am. J. Surg. **54** : 170-184, 1945.
26. ——— Anesth & Analong. **24** : 51-65, 1945.
27. ——— Am. J. Surg. **60** : 335-348, 1942.
28. Cole, W. H., Arch. Phys. Therapy, **24** : 670-675, 1943.
29. Devine, J., M. J. Australia **2** : 476-479, 1943.
30. Sellers, E. A. & Willard. J. W., Canad. M.A.J. **47** : 461-464, 1943.

APPENDIX II.

Note on segregation and treatment of cases of tetanus with a view to carry out clinical trials of V-187 in India on behalf of the Medical Research Council by the Clinical Research Advisory Committee, I.R.F.A.

Objective. -

To make available as large a number of cases of tetanus as possible within a reasonable period of time for clinical trial of V-187.

Preliminary statement.

1. At present cases of tetanus occur sporadically all over the country and are admitted to any hospital available. In these hospitals they may be treated in medical wards, surgical wards, children's wards, and gynaecological and obstetrical wards or infectious diseases wards. Thus at one time a small number of cases will always be present, yet so widely distributed as not to be available for clinical study.

Cases are occurring :

(A) In large towns with hospitals of 200 beds and over.

(B) In country districts with hospitals of under 200 but more than 50 beds.

The total per province may be quite large.

Therefore to make the maximum number of cases available for clinical study it is apparent that there must be :—

A. *In large towns.*

An over-all scheme for segregation of cases in one ward of each hospital, under the charge of the worker carrying out the research.

B. *In all such centres throughout India* there must be :

(a) Uniform treatment.

(b) Uniform bacteriological control.

(c) Uniform recording.

(d) Uniform administrative control.

(e) Direction, co-ordination and inspection of the work by a C.R. Com. member.

(f) Collection of data of cases, their treatment and survival rates for the past 10 years in that hospital.

C. *In country districts.*

Treatment and recording of cases as at large centres, by men trained at such. It is evident that the work must begin at the large centres.

2. In order to carry out the broad scheme laid down in para. 1 above, liberal co-operation by higher authority (S. Gs. and I. Gs.) is essential since in almost every instance the hospitals concerned would be Government ones.

Thus orders from appropriate higher authority would be necessary to :—

(a) Initiate the plan.

(b) Equip and set apart a ward at each centre or hospital for this purpose.

(c) Cause segregation of all cases at centres equipped for this purpose.

(d) In consultation with the I.R.F.A. appoint the person to take charge of the unit and the work.

(e) Assure this person responsible for the work adequate personnel and co-operation with a department of bacteriology.

(f) Permit visits at all times by an appointed I.R.F.A. Inspector for purposes of scientific control of the work in progress.

(g) Ensure co-operation in any administrative steps this Inspector may advise as necessary to ensure that the work may be accurate and of use for the purpose of this study.

N.B.—From a study of local conditions (Calcutta) it is evident that little or no cost would be incurred and no extra personnel would be required. Only a *rearrangement* of *one* small ward and reallotment of equipment and staff is necessary.

DETAILED STATEMENT.

A. *The centre.*

(1) *Direction :*—Professor of Surgery, and/or
Worker i/c Unit & Collection of data.
Surgeon

(2) *Ward :*—In one or more large hospitals in large towns.
A ward set apart and labelled for this purpose.

(a) *Position in hospital :*—Easy reach of O.P.D. and full theatre facilities.

(b) *Size :*—5-10 beds.

(c) *Facilities necessary in ward :*

(i) Means of darkening or dimming light from windows.

(ii) Means of screening each case.

(iii) Stands for each bed for I.V. saline and Intra-Gastric drips; (i.e. two stands per bed).

(iv) Means of sterilization of instruments, bowls and glass-ware.

(v) Arrangements so that nurse on duty can observe all cases easily and does not have to leave the ward except when going off duty.

(vi) Table and box for filling case notes, registers, case sheets etc.

N.B.—It must be stressed again that for the collected data to be of any value at all treatment and diet must have been uniform throughout. Records are valueless unless they represent accurate observations under strictly comparable conditions.

A. *Treatment.*

As treatment throughout India is by no means standardized it is necessary for the purpose of this study to go into considerable detail in order to ensure uniformity.

(a) *Prophylaxis :*—All accident cases throughout India should receive 3·000 International Units of tetanus anti-toxin as soon after wounding as possible.

Thus all cases developing tetanus will have had this dose of anti-toxin.

(b) *Curative :—*

Principle :— (i) Prevention of further absorption of toxin.

(a) Administration of adequate doses of anti-toxin.
(b) Treatment of wound.

(ii) Prevention of exhaustion :

(a) Control of spasms.
(b) Adequate feeding.
(c) Correct nursing.

(i) *Prevention of further absorption of toxin.*

Principle :— To flood the circulation with anti-toxin as soon as the first symptom appears.

Method :— (a) *Administration of adequate dose of anti-toxin.*

(x) *Dose :*—200,000 International Units.

(xx) *Route :*—Intravenously (Intramuscularly only if I.V. not possible).

Precautions :—Anaphylaxis.

(x) *N.B.*—(i) Wound not to be dealt with until at least an hour after the I.V. dose of anti-toxin has been given.

(ii) It has been shown that 14 days after 200,000 units of anti-toxin have been given I.V. in cases of tetanus, as much as 15,000 units is still circulating. Therefore a single large dose, or at most a second large dose 4 days after the first, in a very severe case where wound cleansing could not be adequately carried out, should be sufficient.

(iii) Mere tonic rigidity in a recovering case is not an indication for further anti-toxin.

(xx) For purposes of this investigation anti-toxin should be given only by these routes. The value of the intrathecal route is still disputed and for this reason should not be used here.

(b) *Wound treatment.*

A. *Developed cases.*

Principle .—To eradicate the source of toxin formation as early as possible ; free drainage.

(i) Excision of a small wound en block if possible ; free drainage.

(ii) Wide opening up of others ; all pockets should be converted into well drained *open* shallow spaces. Free drainage.

(iii) Evacuation of all pus ; debris ; foreign bodies ;—Free drainage.

(iv) Free irrigation of wounds not easily opened up widely, with saline or Dakin. Free drainage must be ensured at the same time.

(2) In (ii), (iii), (iv) V.187 would be applied locally in place of any other application or irrigation. Thus two comparative series would be produced.

B. *Presumably infected wounds.*

V.187 locally with debridement only, and prophylactic 3,000 units anti-toxin.

(ii) *Prevention of exhaustion :—*

(a) *Control of spasms :—*

Principles :— To prevent respiratory spasm, and exhaustion from oft repeated spasm.

*Means of control :—*Vary from luminal gr. i, 4 hourly in cases of local tetanus or mild tonic spasm, to full anaesthesia in the developed convulsive case.

Suggested methods in severe cases :—

(i) Avertin, 0·07 c.c. to 0·1 c.c. per kilo of body wt., every 4 to 6 hours as indicated.

(ii) Continuous ½% pentothal drip in N. saline. By means of a two-way attachment to the I.V. needle a 5% solution of pentothal can be injected to control any severe spasm, or to allow handling the patient as may be required in nursing.

The pentothal drip is preferred since it is easy to control and supplies much wanted fluid at the same time. It also gives an easy route for any injection that may be required in addition, leaving the patient quite undisturbed.

With either method oxygen and air given by an injector mask is an advantage.

With pentothal an intramuscular drip of intercostin can be given in the severest cases. Oxygen *must* be given by means of a positive pressure apparatus in this instance.

(b) *Adequate feeding.*

*Principle :—*To maintain full nourishment so that exhaustion cannot occur in spite of the prolonged muscular work entailed by the disease.

*Method :—*Intra-gastric drip via a Ryle's tube through the nose is the simplest means of maintaining an adequate caloric input.

*Solution :—*Whole milk made up with half the quantity normal saline. (Boiled milk may be given without saline).

Rate :—30 drops per minute.

Quantity :—3 quarts of milk in 24 hours.

Caloric value :—2,400 cals. approximately.

The method has been tried out and found very satisfactory in all types of cases. It is particularly indicated in severe spasmodic types and those affecting the muscles of deglutition particularly.

(c) *Correct nursing.*

*Principle :—*As little disturbance as possible with full control and observation at all times.

The above methods of continuous drip feeding and continuous intravenous anaesthesia make nursing very much easier.

Place the patient on a firm mattress on his side so that the light is not on the face. Under the buttocks place a gynaecological pad, the end of which

is led into a bucket beside the bed. Stool and urine are often passed at the beginning or end of a spasm, or the patient may become incontinent. The arrangement allows the faecal matter to be sluiced down with the minimum disturbance to the patient.

The temperature must be watched carefully since hyperpyrexia may be due to heat stroke supervening on tetanus, as well as to the disease itself. Blood slides must be taken also if there is any abnormal rise in the temperature.

The nurse must be thoroughly conversant with the features of tetanus, and with the manipulation of the saline and intra-gastric drips.

C. *Uniform records.*

In order that the collected records may be of the fullest possible use, clinicians engaged in the enquiry should conform to the following minimum. Any other relevant data should, of course, be noted at the same time.

(x) *Series :*

Record : No. Centres

(%) Type Date of admission
Date of death/discharge

Name

Age Sex

History :

Date of wounding Site of wound Nature

Treatment of original wound

Anti-toxin (prophylactic) :

First symptoms and date of development :

of development

Progress and sequence of symptoms before treatment began :

Treatment

Date of injection of anti-toxin and dose and route

Date of first use of V.178.

Method and quantity

Repetition

Cultures taken Date Results
(N.B. Note of standard bact. methods in use required for each centre)

Wound Nature Treatment

Progress & sequence of symptoms after V.187 applied (with dates)

Method of feeding Diet Remarks

Method of controlling spasms with details of success

Notes on nursing

Outcome of case

Post mortem findings

Series A=Developed disease present on admission.

Series B=Prophylactic use of V.187.

(%) Type=Classification of clinical tetanus (See notes attached)

NOTE ON CLASSIFICATION OF CLINICAL TETANUS (after M. COURTOIS-SUFFIT & R. GIROUX).

(The abnormal forms of Tetanus. University of London Press Ltd., and Masson et Cie, Paris. 1918).

Type I :— Generalised classical form in which the patients have prodromal pains of neuralgic type and stiffness, with or without transitory contraction of some muscles in the wounded area. Trismus sets in, followed by contraction of neck muscles and those of deglutition. Facial muscles follow next and ophisthotonus soon after. Now the abdomen and entire body is rigid. Contractures are intermittent but occur on the mildest stimulus. Lastly asphyxia from respiratory muscle spasm kills the patient.

Type II :—Cephalic tetanus.

(1) *Paralytic.*

(a) Facial paralysis with contractures localised to the muscles of head region. May spread and become generalised.

(b) Ophthalmoplegic type after eye wounds.

(2) *Non-paralytic.*

May be simple contractures of face muscles with trismus; pharyngeal spasms, often spreading to respiratory muscles. May commence with convulsive spasms of face and spread rapidly to neck, pharynx and finally diaphragm.

Type III :—Localised tetanus.

A. *Tetanus of limbs.*

(1) *Monoplegic.*

Stiffness of local muscles around wound followed by clonic spasms of the limb as the disease progresses. Abdominals and masseters may be contracted.

(2) *Paraplegic.*

May affect upper limbs or lower limbs. The arms are in forced flexion, the legs in forced extension and equinus.

B. *Tetanus of abdomino-thoracic muscles.*

Abdomino-thoracic groups affected, and may be the masseters, but no others.

C. *Splanchmic tetanus.*

Involves muscles of deglutition and respiration.

Type IV :—Mild or chronic.

Here merely tonic rigidity develops locally or may be more generalised, but general condition remains excellent and no spasms occur. Usually recovers but may lead to any one of the other forms. This is also the condition seen in early convalescence after recovery from tetanus.

APPENDIX III.

REPORT ON THE DISTRIBUTION OF TYPHUS FEVER IN THE ARMY IN INDIA 1943-46.

MAJOR S. L. KALRA, I.M.S./I.A.M.C.
G.H.Q., Medical Research Organisation. (Field Typhus Research).

The statistical data have been obtained from an analysis of typhus cases in the Army in India during the years 1943, 44, 45 and 46 (to August). The information was compiled from A.Fs. A-35 (Notification of Infectious Disease), available in Medical Directorate. Only those cases where the clinical diagnosis of typhus was confirmed by Weil Felix results in diagnostic titre are included.

The division into different sub-groups is based on the assumption that the presence of high OXK agglutinins only is indicative of scrub typhus; high OX2 or high OX2 and low OX19 agglutinins, indicate tick typhus; and high OX19 agglutinins indicate murine typhus. The previous assumption that classical epidemic louse borne typhus existed in India has been based on clinical findings and Weil Felix results, neither of which can definitely differentiate between epidemic and murine typhus.

No strains or Rickettsia prowazeki have so far been isolated. On the other hand murine typhus has definitely been shown to be present in a number of places in India by the isolation of *Rickettsia mooseri* from man and rodents. The presence of murine and the absence of classical typhus has also been confirmed by rickettsial agglutination tests on sera examined since 1944. All cases of high OX19 agglutinins, except those from Kashmere, have therefore been presumed to be murine typhus, and classical typhus is excluded from this discussion.

RELATIVE FREQUENCY OF DIFFERENT TYPHUS SUB-GROUPS IN THE PROVINCES.

(See Table I and Graph I).

Bengal and Assam. (Excluding Manipur Road area and Manipur).

These are the main scrub typhus provinces of India. The incidence is far higher than in any other province, and is six times greater than murine typhus. The majority of murine typhus cases are from the town of Calcutta. The incidence of tick typhus is insignificant.

United Provinces :—The incidence of murine typhus is higher than the others, but all the three types are fairly well represented, although the incidence of the whole of the group is relatively rather low.

But the available data does not in reality present a true picture of the whole province and are representative of the plains only. The present investigation in Kumaon hills has revealed that scrub typhus is as common in these hills as in Assam or Burma. The low figures are due to comparatively fewer troops being stationed in this area.

Punjab :—This is a predominantly murine typhus area, the incidence of the other types being comparatively insignificant. The disease is spread more or less uniformly over the whole of the province. But the type of typhus present in the hills in the north and north-east is not known and needs investigating.

Central Provinces :—The incidence of murine typhus is slightly higher than the others, but all three types have a fairly high incidence. A special feature is the very high endemicity of tick typhus.

Bombay Presidency :—Here again the incidence of murine typhus is the highest. This disease is 3·2 times more common than scrub typhus. Tick and scrub typhus have a more or less equal distribution.

South India :—(Excluding Bombay Presidency).

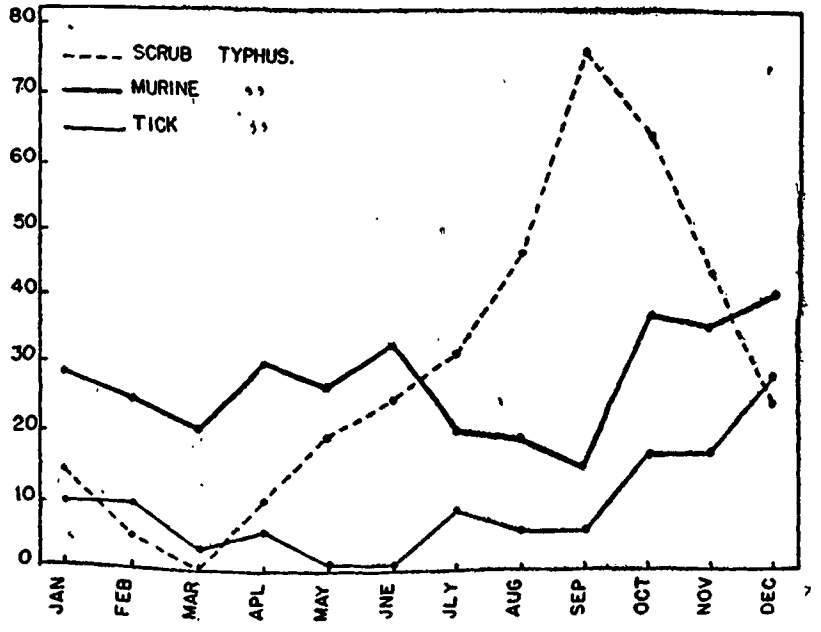
All the three types are represented, but murine typhus has the highest incidence with scrub typhus next.

Of the islands near India, scrub typhus has been reported from the Andaman and Nicobar islands.

GRAPH I

SEASONAL INCIDENCE OF TYPHUS IN INDIA.

(AVERAGE OF 3 YEARS)



Considering the whole of India (Graph I) the various types in order of importance are scrub typhus, murine typhus and tick typhus; but if Bengal and Assam are excluded murine typhus is the chief problem in the rest of India. Tick typhus from the available evidence is of the least importance. Firstly, it has the lowest case incidence, and secondly, the rickettsial strains are not highly virulent. Up to the present two strains have been isolated, one from a patient in Poona (by Major Seaton) and the other from the tick-Haemaphysalis leechi var. *indica*—in Imphal (by the author). Although both these strains showed some antigenic relationship to *Rickettsia rickettsi* the causal organism of Rocky Mountain Spotted Fever, they produced very mild symptoms in guinea pigs, and died out after a few passages. Kumaon strains, though not yet confirmed, are behaving similarly.

TOPOGRAPHICAL DISTRIBUTION.

Scrub typhus.

Scrub typhus is highly endemic in the humid regions of the plains of Bengal and Assam, and through the lower Himalayan range from Assam to Kashmere. The average annual rainfall in these areas is over 40 inches and the area is more or less bounded by an isothermal line of mean maximum temperature of 87·5° F. for the month of June. In the Kumaon range, heights up to 8,000 ft. have been investigated and Trombiculid mites have been found. *T. deliensis* is present in abundance at heights of 7,000 ft., and *R. orientalis* has been recovered from these mites. The climate in these hills is tropical and subtropical from the foothills to 4,000 feet, while above this up to 11,000 feet it is temperate. The gradation of forest as one ascends to the higher altitudes is mixed deciduous and evergreen up to 3,000 to 4,000 feet, while above this up to 8,000 feet the main forest trees are pines, yew, cypress, oaks and low level silver fir. In pine areas the ground is covered with a dense crop of grasses and widely scattered shrubs, but associated with other types, particularly oaks, there is an extensive ground floors of herbaceous plants with moist soil which provides good foraging for rats and a suitable habitat for mites.

Murine typhus.

This is mainly an urban disease. Rats trapped away from habitations are seldom infested with fleas. Although murine typhus occurs in almost every climate and is more uniformly spread over the whole of the India than any other type its highest incidence is in arid and semiarid regions. Those areas with a rainfall of 20 to 40 inches or less, have open dry forests of stunted and often thorny trees, as found in the drier parts of Central India and Deccan, Southern Districts of U.P. and the plains of the Punjab and Sindh.

SEASONAL INCIDENCE (*See* Graph II).

(a) *Scrub typhus.*

Scrub typhus occurs all the year round, but the peak period is from July to November. This seasonal incidence is similar to that found in most other countries where scrub typhus is prevalent. In the greater part of these areas, from June to September is the period of maximum rainfall, high temperatures, and luxuriant vegetation, providing directly and indirectly suitable conditions for a rapid increase in the mite population and a consequent rise in the number of cases.

(b) *Murine typhus* has the highest incidence in the last three months of the year; probably the rats migrate towards the houses in the colder months, consequently increasing the chances of flea bites. In the Middle East the highest incidence is in the summer months of July, August and September but not in winter.

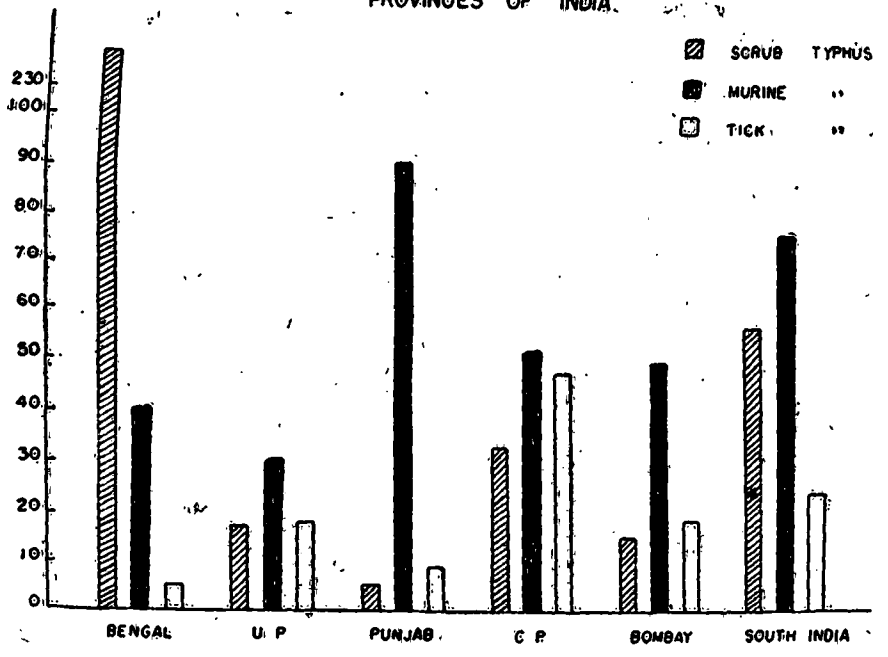
A study of the seasonal variation in the rat-flea population may furnish a clue to this difference in seasonal distribution.

(c) *Tick typhus* also shows a definite rise in the last quarter of the year, even more marked than murine typhus. Up to the present time no adequate reason has been found to explain this rise. More data must be known about the ecology of the vector species in India; that available would appear to indicate

that contrary to observations in America, the ticks in India do not hibernate in winter. The probable reason being that the Indian winter is not as severe as the American.

GRAPH II

RELATIVE INCIDENCE OF TYPHUS IN DIFFERENT PROVINCES OF INDIA.



TYPHUS CASES IN DIFFERENT PARTS OF INDIA.

TABLE I.

Province.	Scrub typhus.	Murine typhus.	Tick typhus.
Bengal	237	40	5
United Provinces	17	30	13
Punjab	5	90	9
Central Provinces	33	51	47
Bombay Presidency	15	49	18
Madras and Mysore	56	75	24
All India	363	335	116
All India less Bengal and Assam ..	126	295	111

The figures in the above table are total figures. The results of different provinces are, therefore, not comparable with each other only the relative incidence of different types is of value.

APPENDIX IV.

A meeting to draw up conjoint research on cirrhosis was held in the Haffkine Institute, Bombay, at 7 P.M. on the 9th December, 1946.

The following were present :—

Dr. V. R. Khanolkar. (*Chairman*).
Major A. T. Andreasen.
Major H. Lehmann.
Prof. T. Bhaskara Menon.
Dr. P. V. Gharpure.
Dr. V. N. Patwardhan.
Dr. A. Das.
Prof. S. W. Hardikar.
Dr. M. V. Radhakrishna Rao.
Prof. V. R. Naidu.
Dr. B. B. Dikshit.
Dr. R. W. Linton.

1. It was decided that it would be of advantage to coordinate the research on infantile cirrhosis on some definite and restricted lines. In view of the fact that the clinical picture of infantile cirrhosis has much in common with the fat disturbance variant of the trophopathic cirrhosis (in the nomenclature of Himsworth and Glynn) which is amenable to choline treatment, work should be concentrated on choline therapy for the coming year.

2. The early stages of the disease should be more clearly defined and if possible liver function tests should be performed together with clinical observations. As a check on these observations, liver function tests and liver biopsies should be done wherever possible and the specimens investigated histologically and biochemically. These liver biopsies should not only be done in the early stages of the disease; but should be continued throughout the later stages to obtain a histological and biochemical background for the evolution of the disease.

3. (i) In view of these decisions Dr. Radhakrishna Rao's future programme of work was recommended for one year in the first instance as follows: Investigation of the lipotropic factors, specially choline in the production of experimental cirrhosis.

(ii) Professors V. R. Naidu and V. Subrahmanyan's enquiry for one year to carry out the following clinical investigations:

(a) observations on the value of choline chloride as a therapeutic measure on cases of infantile cirrhosis;

(b) liver function tests at various stages of the disease, particularly thymol turbidity test;

(c) perform liver biopsies with a view to investigating the histological and biochemical alterations in the liver and the possible role of bacteria in the etiology of cirrhosis.

(iii) Prof. Kutumbiah's proposals were recommended for one year and it was pointed out that it may not be sufficient to determine the choline content of the diet alone, but also the actual absorption of the dietary choline and to investigate choline metabolism in affected and normal children.

(iv) Prof. Bhaskara Menon's proposal was recommended for one year, during which time it was anticipated the effect of repeated administration of castor oil or other oils, as practised in different parts of India, could be established by experiments on sucklings.

(v) Prof. Das's proposals were not recommended.

APPENDIX V.

REPORT OF THE 2nd MEETING OF THE SUB-COMMITTEE APPOINTED TO DRAFT TENTATIVE PROPOSALS FOR THE STUDY OF LATHYRISM IN THE CENTRAL PROVINCES & BERAR.

The Committee met at 10 A.M. on the 8th December, 1946 in the Haffkine Institute, Bombay.

PRESENT.

1. Major A. T. Andreasen.
2. Major P. D. Bhave.
3. Dr. S. W. Hardikar.
4. Dr. V. R. Khanolkar.
5. Dr. (Miss) Kamala Bhagvat.
6. Dr. V. N. Patwardhan.

The Committee considered the draft note sent by Dr. Khanolkar (Appendix A) and the notes regarding clinical and biochemical investigations sent by Dr. Hardikar, Dr. Patwardhan and Major Bhave. They also discussed the points which needed clarification, stated in Appendix to the report of the 3rd meeting of the Clinical Research Advisory Committee held in New Delhi on the 22nd July, 1946.

The Sub-Committee suggested as follows:—

1. In any scheme which may be adopted in connection with this investigation, it is recommended that the Government of the Central Provinces & Berar should undertake the financial responsibility and provide the personnel required for field and clinical investigations. The expenditure on equipment, field laboratory and expenditure in connection with the personnel for laboratory investigations and experimental work should be a charge on the I.R.F.A.

2. It is expected that the probable duration of this enquiry would be about three years and the plan of work has been so outlined that the experience gained during the first year will help the workers to tackle the problem more directly in succeeding years. The Committee, therefore, recommend that the Government of the Central Provinces & Berar should entertain a scheme for the continuation of this project, so far as it relates to them, for a similar period of three years.

3. The Committee recommend that as far as possible the investigation should, during the first year, be carried out on all the three stages of the disease (incipient, mild and grave) and as near as possible to 100 cases of each type should be investigated in accordance with the plan given below. It is expected that the co-operation of the Government, district, as well as Mission, hospitals in the Province would be made available for carrying out this investigation through Government agencies.

4. A perusal of the literature on the subject shows that since 1933, studies on lathyrism have been in progress in Spain and the Public Health Commissioner with the Government of India may be requested to obtain through Government sources, detailed information regarding this work. If possible, reprints of the original articles published by the Spanish workers may also be obtained. Abstracts of these articles have appeared in "Nutrition Abstracts & Reviews".

5. The Committee consider it essential to study post-mortem material available from cases of lathyrism. They therefore recommend that the Government of the Central Provinces & Berar should issue necessary instructions to facilitate post-mortem examination in cases known to have suffered from this disease. These instructions contained in Appendix A to this report should be made available to all District Medical Officers in the areas in which lathyrism is prevalent.

6. The Committee suggest that in the appointment of the clinical, field survey and laboratory staff, it is absolutely essential to employ trained and experienced persons. The appointment of inadequately trained personnel would result in a complete failure of the plans now submitted.

The forms recommended (Appendices D and E) are not intended for wide circulation to the Medical Officers in the Province, but have been designed to guide and help trained men with a view to systematizing their ideas and uniformity in the collection and recording of data.

7. The field surveys and clinical investigations detailed above may be supplemented by an investigation on experimental animals for the purpose. The investigations in progress at Coonoor and Dacca may be continued and the search for a suitable animal, in which lesions similar to those in man could be produced should be continued. On the basis of the first year's experience, a definite line of experimental work can be laid down at the end of the first year.

Pharmacological experiments on *Lathyrus sativus* and *Vicia sativa* should be carried out separately to discover the nature of poisonous action, if any, and later followed up by attempts to isolate the poision.

8. The Committee considered the proposal submitted by Dr. M. N. Rudra of the Dharbhanga Medical College, Laheriasarai. The details supplied in regard to the line of work to be undertaken did not satisfy the criteira laid down for submission of proposals for work by the Secretary, I.R.F.A. The Committee do not, therefore, recommend a grant for this enquiry.

APPENDIX A.

Technique for removal of the brain and spinal cord.

Brain.

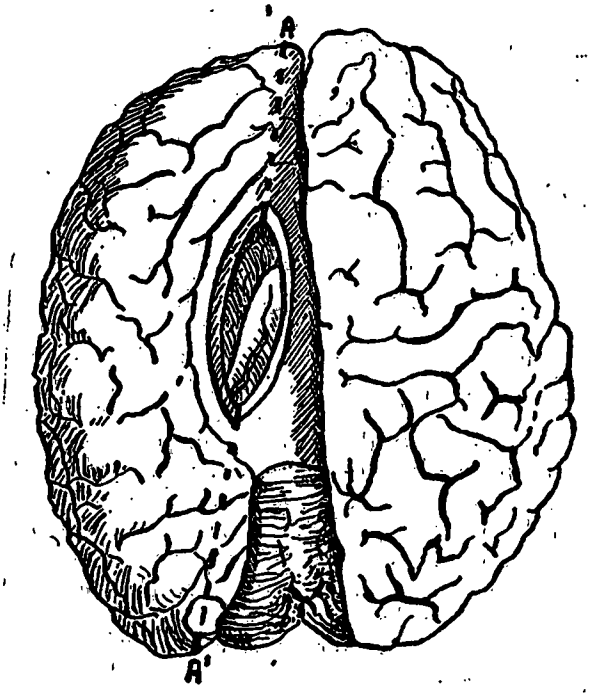
The incision in the skin of the head passes down to bone from side to side over the vertex to the mastoid processes on either side. The scalp flaps are then stripped forwards and backwards; the forward flap being stripped to the level of the orbits and the posterior flap to the lower border of the occipital bone.

The bone incision is made a little above the level of the eyebrows through the frontal bone and continuing backwards through the squamous part of the temporal bones to the mastoids in such a way that, if it completely encircled the head, it would pass through the occipital bone farthest from the root of the nose. These two points are joined by an oblique cut passing across the back of the skull through the sagittal suture at the junction of its anterior two-thirds with its posterior third. Care should be taken not to damage the dura with the saw. Having cut through the bone, the skull cap should be gently raised from the dura by the use of an " L " shaped chisel and, if necessary, a periosteal separator may be used.

After removing the skull cap, the superior longitudinal sinus should be opened and examined. The dura is then cut round at the same level as the bone; the falx cut through its anterior attachment and the brain removed by gently raising the olfactory nerves and cutting through the others; also cutting the tentorium cerebelli at its attachment to the petrous portion of the temporal bone and cutting through the uppermost part of the cervical cord. The brain is first examined at the base. The arachnoid covering and the content of the basal cisterns should be carefully examined. Dissect carefully the main arteries. Transverse incisions are made through the medulla and pons. When these have been examined the brain should be turned over and the two lobes gently drawn apart and the corpus callosum incised from front to back so as to open the lateral ventricle on each side, and incisions are made through the walls of the lateral ventricles throughout their length so that the brain opens out like a book. Transverse incisions should then be made through caudate necleus, internal capsule, optic thalamus and lenticular necleus and, if these are made at the same level on each side, one side can be compared with the other. A vertical incision is made through the long axis of the vermis, so as to open the fourth ventricle and one horizontal incision, passing from the midline through each lobe of the cerebellum and a number of smaller vertical incisions are made through the dentate nuclei. The lateral sinuses are opened up throughout their length. (Diagram of incision appended).

The brain stem.

The crura, pons and medulla are examined by a series of transverse incisions and the stem is kept for fixation and study along with the spinal cord as described below. As a rule, three incisions through the pons and three through the medulla are sufficient. The knife should be sharp, as clean sections are essential for further study. To remove the pituitary the dura covering the sella turcica should be incised Antiro-Posteriorly and the posterior clinoid processes should be carefully chiseled away and the two flaps carefully raised. Unless these



The left hemisphere is drawn to the left so that the corpus callosum is exposed and the incision is made laterally along the line A—A' with a long, thin, narrow knife. A similar incision is made on the right side.

precautions are taken the anterior lobe will be damaged and the posterior lobe ruined.

Spinal cord.

The removal of the spinal cord is a laborious rather than a difficult procedure. The body should be turned on to its face and the chest should be supported by a block so that the back is lightly arched and the head hangs down. It is important, however, to avoid too much arching as this produces tension on the cord and may damage it. The incision is made in the mid-line down the back, extending from the head to the sacrum. Hold the knife firmly, cut with its belly, not with its point, and prevent the skin from slipping laterally under the blade by placing the thumb and fingers of the left hand astride the back of the knife, thus pressing firmly on the skin at each side whilst the incision is made. The skin and subcutaneous tissues are reflected on either side. Reflect the muscular masses on each side from the spines and laminae of the vertebrae and from the lower part of the occipital bone. The laminae should be well cleared of muscle and tendon as far outwards as the projections of the transverse processes. Unless a clear view is obtained of the lamina in this way, it is impossible to make accurate saw cuts, and, unless these are made, either the cord will suffer or it will be extremely difficult to get a satisfactory exposure. The saw cuts are through the laminae as far from the mid-line as possible without involving the canals from which posterior roots emerge. A simple hack-saw (Hay's) is the most effective instrument for this purpose. The easiest place to begin the saw cuts is in the dorsal region, and the saw blade should slant slightly from without towards the mid-line. All the laminae from the first cervical to the last lumbar should be cut through. Saw gently and stop immediately the laminae are completely divided ; this can be gauged by the resistance offered to the saw. (The arches of the atlas and axis are best removed with bone forceps). Bone forceps are then used to remove the laminae, and it is important if the bone forceps are used that pressure should only be made upon bone surfaces and never upon the cord. If the saw cuts have been satisfactorily made the whole of the posterior part of the arches will come away in one long ribbon and very little cutting will have to be done with bone forceps. Further, the cord will be exposed throughout its length ; its membranes and nerves will be intact and undamaged. To remove the cord, a pair of Spencer Wells or Kocher forceps should be attached to the dura covering the cauda equina ; then with ordinary dissecting forceps gently pull the dura to one side, while cutting through the nerve roots with a sharp scalpel. When this is completed, the attachment of the dura to the posterior aspect of the skull can be cut through with a scalpel. Next cut through the dura and the nerves distal to the Spencer Wells, and holding the Spencer Wells gently raise the cord from its bed, cutting through any attachments to the posterior surfaces of the bodies of the vertebrae. The cord should not be pulled, but it should be kept straight and taut during this manoeuvre. It is most important that the cord should not be bent or otherwise pressed as post-mortem damage to a cord may easily obliterate any traces of ante-mortem disease.

The dura can now be cut through its lateral and anterior attachments to the skull. The cord will now be free and can be removed for examination. It should be laid on a tray or a table and the posterior surface of the dura incised in the mid-line with scissors. If this is done carefully, the pia arachnoid will be left intact enclosing fluid or other content. The cord and its membranes

should be examined and changes noted. Transverse sections are now made through the cord at two levels (Cervico-Dorsal ; Dorso-Lumbar). These sections must be made with a very sharp moist knife, and should leave the dura intact on one side. The sections form convenient hinges through which the cord may now be bent for putting into fixative.

APPENDIX B.

Technique of preservation of the specimens

Before fixation one should make it a routine to describe briefly the appearance of the dura mater and its sinuses, which have been opened, the pia-arachnoid, the vessels at the base and the configuration of the gyri. For the fixation of the brain and spinal cord formaline solution (37% formalde-hyde) is recommended. The formaline should be preserved in brown bottles with about ½ inch layer of powdered chalk (calcium carbonate) at the bottom in order to neutralise any formic acid which is usually formed on keeping. The formaline solution is made by mixing one part of formaline with nine parts of a 0·9% solution of sodium chloride in tap water. *The volume of the fixative should in no case be less than twenty times the volume of the specimen to be fixed.* The tissue should not be pushed or forced into bottles and should not be allowed to touch the glass sides of the vessel in which it is being fixed. It is kept away from the glass by layers of cotton wool in the fixative. The time for fixation will depend on the thickness of the specimen, 3 days to one month ; 2-3 days will suffice for a specimen under an inch in thickness. The fixing fluid must be changed after 24 hours and again after 4 days whenever possible.

Note 1 :—Proper fixation of the whole brain with the cord is a procedure which demands a certain amount of experience and adequate laboratory facilities. It may, therefore, be advisable to concentrate on proper preservation of the brain stem and the cord, as it will not be necessary to have very large jars and large amounts of fixing fluid for this.

Note 2 :—Tissues for histological examination should never be allowed to dry in a tray or immersed in pure formaline or left in water or saline.

APPENDIX C.

Instructions for sending the material to the pathologist.

If the specimen is small, it can be sent in a bottle filled with 5% formaline saline, tightly closed and sealed with paraffin wax. If it is a big specimen it is fixed in 10% formaline saline for several days, removed and packed in a tin between layers of cotton wool or tow, soaked in 5% formaline saline solution. It is essential that the specimen is not squeezed, pinched, pressed or torn at any stage during all processes described above. When handling tissues for examination hold the part or the connective tissue with the forceps well away from the portion selected. The tin is then soldered to make it water tight.

APPENDIX D.

Index Reference Number :—

LATHYRISM ENQUIRY.

CASE SHEET.

No blanks to be left. All information should be entered including a definite negative.

Name of medical officer

Date Place (village & district) of enquiry.

SECTION I.

Name of patient Address
Age Sex Occupation
Provisional diagnosis
Final diagnosis and remarks (To be filled in by the Officer-in-Charge of the enquiry)

(A) History and nature of present illness
Time of onset
Mode of onset
Number and dates of remissions and relapses if any

(B) Personal history and habits
(1) Alcohol
(2) Opium
(3) Tobacco
(4) Bhang, ganja etc.
(5) Any other habit producing drug.

(Note :—Note the form in which used, frequency and quantity).

(C) History of serious previous illnesses with particulars of treatment.

(D) Family history
Other persons affected with lathyrism, with particulars.

(E) Other cases of lathyrism in the village with particulars.

SECTION II.

Physical examination Date

Sitting height in inches Weight (pounds)

(A) *General inspection.*

(i) Skin & mucous membranes :—

Ringworm.	Anaemia.	Phrynoderma.
Scabies.	Edema.	Pellagrous dermatitis.

Pediculosis. Ulcers. Xerophthalmia.
Bitot spots.
Gingivitis.
Angular stomatitis.
Fluorosis (Teeth).

(ii) Deformities of skeleton.
Head.
Chest.
Limbs.
Pelvis.

(B) *Alimentary system.*

(i) State of appetite and digestion.
(ii) Bowels (constipation/diarrhoea).
(iii) Liver.
(iv) Spleen.

(C) *Circulatory system.*

(i) Heart.
(ii) Arteries.
(iii) Pulse.
(iv) Blood pressure.

(D) *Respiratory system.*

(E) *Genito-urinary system.*

(i) Potency.
(ii) In females particulars regarding :—
Menstrual periods.
Number of children.
Miscarriages.

(F) *Locomotor and nervous system.*

Locomotor.

(i) Condition of muscles.
Wasting.
Wrist drop.
Foot drop.
Gait.
Paresis.
Paralysis.
Spasticity.
Tremors.

(ii) Condition of joints.
Swelling.
Stiffness.
Limitation of movements.

Nervous.

(i) Any obvious signs of mental or emotional derangement.
(ii) Eye.
Night blindness.
Eye movements.
Light reflex.
Visual fields.
Fundus examination.

(iii) Cranial nerves.

(iv) Spinal nerves.
Sensory disturbances.
Motor disturbances.

(v) Coordination of movements.

(vi) Reflexes.

Superficial.	Epigastric.	Cremasteric.	Babinski.
Deep.	Knee jerk.	Ankle clonus	Triceps. Supinator.
Control of Sphincters.		Bladder.	Rectum.

(vii) Vasomotor disturbances.

Flushing.	Dryness.
Sweating.	Syanosis.

(G) Note on treatment received up-to-date.

Index reference No.

INVESTIGATION ON LATHYRISM.

Diet chart.

A. *Intake of foodstuffs.*

Name of patient.....

Age.....Sex.....Religion, caste & community.....

Occupation.....

Number of members in family

Adults.
Males.
Females.
Children under 12.

Notes.—1. It may not be possible to obtain figures for individual consumption in a family. The information for the total intake of the family should be obtained and the data converted into adult consumption units.

2. The quantities of articles consumed should be entered in lbs. and ozs. Local weights and measures should not be entered.

Cereals.

Rice.
Wheat.
Maize.
Jowar.
Bajiri.
Ragi or nachni.
Any other.

Pulses.

Bengal gram.
Red gram.
Green gram.

Black gram.
Khesari or Teora (*Lathyrus sativus*).
Akta (*Vicia sativa*).
Other legumes.

Vegetables.

Fruit vegetables.
Root vegetables.
Leafy vegetables.

Fruits.

Sugar.
Jaggery.
Fat.
Oil.
Ghee.

Animal food.

Meat.
Fish.
Eggs.

Milk.

Cow or buffalo.
Any other articles of diet not included in the above list.

B. *Dietary intake of nutrients per adult unit per day.*

Total calories.	
Carbohydrates.	gms.
Proteins.	,,
Fats.	,,
Calcium.	mg.
Phosphorus.	,,
Iron.	,,
Vitamin A value	I.U.
Thiamin.	mg.
Riboflavin.	,,
Nicotinic acid	,,
Ascorbic acid.	,,

Laboratory investigations.

I. Clinical laboratory investigations.

1. Blood.

(a) Cytological examination.
R.B.C., W.B.C., total count.
W.B.C. Differential count.
Haemoglobin in grams per 100 c.c. (only certified instruments should be used).

(b) Serological examination :—Kahn test.

2. Cerebrospinal fluid :—Cell counts.

3. Urine :—Routine examination (must include examination of the urinary sediment).

4. Faeces :—Parasites and ova (concentration method).

QUANTITATIVE BIOCHEMICAL INVESTIGATIONS.

1. (a) Whole blood.
Chlorides.
Sugar.
Non-protein nitrogen.

(b) Serum protein, albumin, globulin.
Serum calcium.
Vitamin A.
Pyruvic acid.
Inorganic phosphorus.
Serum phosphatase (alkaline).

2. Cerebrospinal fluid.
Chlorides.
Sugar.
Protein.

3. Urine.
Glycuronates.
Ethereal sulphates and acetyl compounds.

APPENDIX E.

Index reference No.

SUMMARY AND FOLLOW-UP SHEET.

Name of patient.

Summary of case.

Follow-up. Date.	Notes.

REPORT OF THE MATERNITY AND CHILD WELFARE ADVISORY COMMITTEE.

The meeting was held at 2-30 P.M. on December 10th, 1946, in the Haffkine Institute, Bombay.

PRESENT.

Dr. J. Jhirad. (*In the chair*).
Dr. A. Benjamin.
Miss M. Craig.
Dr. N. A. Purandare.
Dr. Muktha Sen.

Colonel E. Cotter, I.M.S., Secretary of the Scientific Advisory Board, Indian Research Fund Association, kindly attended the meeting.

The following were also present:

Lt.-Col. C. K. Lakshmanan.
Major M. Jafar.
Mr. W. H. Kirby.
Dr. H. Machedo.
Rao Bahadur Dr. K. C. K. E. Raja.
Major B. N. Khan.
Dr. R. G. Dhayagude.
Mr. T. Gonsalves.
Mr. K. K. P. Narasingha Rao.
Dr. B. C. Das Gupta.

1. The question of publication of Dr. Ghosh's reports on (a) Infant Mortality and (b) Standards of Maturity of Indian Infants was discussed. Colonel Cotter suggested, and the Committee agreed, that these two reports should be published by the Public Health Commissioner, as reports prepared by Dr. Ghosh while she was Professor of Maternity and Child Welfare at the All-India Institute of Hygiene & Public Health, Calcutta.

2. The Committee discussed the present unsatisfactory position in regard to the extreme inadequacy of women doctors engaged in public health work and emphasised the need for stimulating active interest among them in preventive health work and qualifying themselves for it. The paucity of such trained personnel is an important reason for research in maternity and child welfare problems not having made adequate progress in the country. The Committee felt that the emoluments offered should be more attractive if women doctors are to be encouraged to take up preventive work for women and children. The prospects which private practice and service in the medical departments of Governments offer to them are so attractive that, without a definite improvement of the salaries now given for work in the preventive field, suitable women doctors will not be forthcoming.

3. *To consider the progress of work on enquiries sanctioned during the year* 1946-47 :

(i) The Committee noted that it has not been possible to start the enquiry into anaemia in pregnancy in the Singur Health Unit under the All-India

Institute of Hygiene & Public Health, Calcutta. They also noted that Dr. Muktha Sen of the All-India Institute of Hygience & Public Health, proposed to carry out the enquiry during the coming year. They recommended a budget grant of Rs. 10,550 for this enquiry. The budget includes provision for a pay of Rs. 300 p.m. for a medical officer in the grade of Rs. 270-15-360-20-400, who will replace the health visitor originally included in the budget.

(ii) The Committee, after hearing Dr. B. C. Das Gupta's report on the work done during the year on the enquiry into the bearing of premature and immature births on infant mortality, recommended that the enquiry be continued during the coming year and that a grant of Rs. 13,031 be provided for it.

(iii) The Committee after reading the note on the work done on the enquiry into the pelvic type and its relationship to body-build in Indian women under Drs. S. K. Basu, S. Mitra and S. Mukerji at the Carmichael Medical College, Calcutta, recommended that the enquiry be continued during 1947-48. The Committee reiterated the view expressed last year that this research has high academic value and that it is likely to have a bearing on practical problems. The investigation should, however, be carried out mainly with the help of the machinery and personnel existing in the teaching institutions, and the Committee, therefore, recommended a grant of Rs. 2,500 to meet the cost of x-ray plates and contingencies during 1947-48.

4. *New proposals :*

The Committee recommended that a grant of Rs. 6,505/- be sanctioned for an enquiry on anaemias in pregnancy under Dr. R. G. Dhayagude, Professor of Pathology, Seth G. S. Medical College, Bombay.

The Committee also recommended that a grant of Rs. 5,384/- be sanctioned during the coming year for an enquiry into the reproductive patterns of urban and rural population under Dr. C. Chandrasekaran, Professor of Vital Statistics and Dr. Muktha Sen of the All-India Institute of Hygiene & Public Health, Calcutta.

5. The Committee discussed at length the draft which has been prepared by Dr. S. Pandit summarising the findings of investigations into the causes of maternal mortality in India. It recommended that Rao Bahadur Dr. K. C. K. E. Raja should modify certain portions of the draft in consultation with Dr. S. Pandit after which it should be printed and circulated.

REPORT OF THE MEETING OF THE FILARIASIS ADVISORY COMMITTEE HELD ON THE 21st JULY, 1946.

MEMBERS PRESENT.

Major-General Sir Gordon Covell. *(Chairman).*
Dr. B. B. Dikshit.
Lieut.-Colonel C. L. Pasricha.
Dr. S. Sundar Rao.
Major R. Senior White.
Dr. C. G. Pandit. ... *(Secretary).*

IN ATTENDANCE.

Lieut.-Colonel A. N. Chopra.
Major A. N. Duggal.
Major M. Jafar.

1. Dr. Sundar Rao gave a comprehensive review of the work done by him at the School of Tropical Medicine, Calcutta, with special reference to problems in human filariasis. Dr. C. G. Pandit gave a short account of work on animal filariasis carried out in several laboratories in India, with special reference to the life history of the parasite.

2. Recent work on filariasis carried out in the United States e.g. early symptomatology and diagnostic procedures was briefly reviewed by Dr. C. G. Pandit. The Committee also considered the valuable report on the Medicinal Treatment of Filariasis Bancrofti by Owsei Temkin, issued by the National Research Council, Washington, U.S.A.

3. The Committee considered Section 3 of the minutes of the third meeting of the Medical Research Liaison Committee for India held on 18th March, 1946 dealing with chemotherapy of filariasis and recommended that new drugs should be investigated in India according to the method suggested by Dr. Hawking. The Committee considered that the School of Tropical Medicine, Calcutta, would be a suitable centre for this purpose in view of their previous experience in this line of research.

4. (a) While considering the future lines of research in filariasis, the Committee was of opinion that it would be advisable to undertake control measures in some selected areas to demonstrate the efficacy of such methods in the prevention and control of filariasis. It was agreed that it would be necessary to carry out such preventive measures for a period of not less than five years.

(b) The Committee also recommended that an enquiry should be instituted to evaluate the intradermal and other immunological tests in the diagnosis of early filariasis.

(c) The Committee noted the suggestion of Dr. Dikshit that the value of changing the physical properties of drugs by wetting agents should be investigated and recommends that work on these lines may be undertaken. Dr. Dikshit agreed to investigate the possibilities of carrying out this work under his direction.

5. The Committee considered a letter from the Director, School of Tropical Medicine, on the question of continuing the filariasis enquiry at the school on

retirement of Dr. Sundar Rao. The Committee recorded their appreciation of the valuable work done by Dr. Sundar Rao during the last 25 years. The Committee noted that the enquiry will be conducted in the Helminthological Department of the School under Dr. Mukerji and that the services of Dr. Bhaduri would be made available to the enquiry as a full time worker. The Committee recommends that the proposal of the Director should therefore be accepted.

6. The Committee recommended that Mr. M.O.T. Iyengar should be made a member of the Filariasis Advisory Committee.

REPORT OF THE MEETING OF THE FILARIASIS ADVISORY COMMITTEE HELD ON 9th DECEMBER, 1946, IN THE HAFFKINE INSTITUTE, BOMBAY.

MEMBERS PRESENT.

Major-General Sir Gordon Covell. (*Chairman*).
Professor R. G. Dhayagude.
Dr. B. B. Dikshit.
Dr. M. O. T. Iyengar.
Lieut.-Colonel H. W. Mulligan.
Lieut.-Colonel C. L. Pasricha.
Dr. C. G. Pandit. (*Secretary*).

IN ATTENDANCE.

Lieut.-Colonel M. K. Afridi.
Dr. A. N. Goyle.
Dr. V. R. Khanolkar.
Dr. K. V. Krishnan.
Dr. T. Bhaskara Menon.
Mr. P. Bruce White.

1. Lieut.-Colonel C. L. Pasricha presented an account of the proposed lines of work on filariasis under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta. The Committee noted that Dr. Bhaduri will be mainly in charge of the work. The Committee also noted that the appointments of one senior and one junior research worker had not yet been made. The Committee recommended the continuation of the enquiry next year and approved the lines of laboratory work as suggested. The Committee noted that the arrangements for field work had not yet been made. The Committee further emphasised that control work on filariasis, if to be undertaken at all, should be on the basis of a long term programme only.

2. The Committee considered and approved the proposal submitted by the Director, King Institute, Guindy on immunological tests in early filariasis.

3. The Committee considered the extract from the minutes of the 5th meeting of the Medical Research Liaison Committee for India held on 10th September, 1946 at the India Office, London. The Committee noted that the Colonial Medical Research Committee had constituted a Helminthological Sub-Committee in relation to the study of systematic helminthic infections inlcuding a long term study of filariasis. The Committee would be very much interested to learn further developments in this subject.

4. The Committee considered a letter from Dr. Dikshit regarding his inability to arrange immediate investigations on the subject of changing the physical properties of drugs by wetting agents. The Committee also noted Dr. Dikshit's suggestion of instituting research into the subject in co-operation with Dr. Venkatraman, Director of the Department of Chemical Technology, University of Bombay. The Committee requested Dr. Dikshit to submit definite proposal in this matter.

MINUTES OF THE 2nd MEETING OF THE INDUSTRIAL HEALTH ADVISORY COMMITTEE, HELD IN NEW DELHI, ON 30th APRIL, 1946.

MEMBERS PRESENT.

Colonel E. Cotter, C.I.E., V.H.S., I.M.S., Public Health Commissioner with the Government of India. (*Chairman*).

Lt.-Col. C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. Jean M. Orkney, W.M.S., Professor of Maternity & Child Welfare, All-India Institute of Hygiene & Public Health, Calcutta.

Professor K. Subrahmanyan, B.E., C.E., S.M., M.I.E., A.M.I.C.E., Professor of Sanitary Engineering, All-India Institute of Hygiene and Public Health, Calcutta.

Dr. V. N. Patwardhan, M.Sc., Ph.D., A.I.I.S.C., Assistant Professor of Biochemistry, G.S. Medical College, Bombay.

Gordon Peace Esq., O.B.E., F.R.I.C., F.R.A.S., Chief Adviser on Factories to the Government of India, Department of Labour, Imperial Secretariat, New Delhi—*vice* Mr. M. T. Raju, I.C.S., Deputy Secretary to the Government of India, Department of Labour.

Dr. J. N. Ray, Ph.D., D.Sc., Director of Drugs & Dressings, Office of the Controller of Purchase (Medical Division), Industries and Supplies Department, New Delhi.

Dr. M. N. Rao, M.B.B.S., M.P.H., Dr. P.H., Assistant Professor of Physiological & Industrial Hygiene, All-India Institute of Hygiene & Public Health, Calcutta. (*Secretary*).

BY INVITATION.

Dr. Thomas Bedford, Ph.D., D.Sc., Industrial Health Research Board, Medical Research Council, London.

IN ATTENDANCE.

Major T. C. Puri, I.M.S., Deputy Public Health Commissioner with the Government of India.

Mr. N. S. Mankiker, B.Sc., B.E., Deputy Chief Adviser on Factories, Government of India, Department of Labour, New Delhi.

Major J. R. Sen, Officer on Special Duty (Industrial Health), Office of the Director-General, Indian Medical Service, New Delhi.

Mr. T. Gonsalves, B.A., LL.B., Assistant Secretary, I.R.F.A.

Dr. M. N. De, M.B., M.R.C.P. (Lond.), F.N.I., F.S.M.F., Professor of Medicine, Medical College, Calcutta, Major E. L. Jones, I.M.S., Additional Deputy Director-General (Social Insurance), I.M.S., New Delhi, Mr. S. C. Joshi, M.A., LL.B., Chief Labour Commissioner, Government of India, Deparment of Labour, New Delhi, and Rao Bahadur Capt. M. G. Kini, M.C., M.B., M.Ch., F.R.C.S.E., Superintendent and Professor of Surgery, Stanley Medical College, Madras, were unable to attend.

The Chairman introduced Dr. Bedford who had come to India to advise the Government of India on industrial health and informed the members of the Committee that the meeting has been convened specially to enable the members to have the advantage of a talk with him.

Dr. Bedford then gave the Committee the opinions he had formed during an extensive tour of 10 weeks visiting factories all over India. He stated that his experience has shown him that living conditions of the vast majority of workers in industries in India were appalling and that there was complete indifference on the part of employers on this score. The workers themselves were apathetic and this was due to the lack of primary education which was the corner stone of public health education.

In a good proportion of the industries visited the construction of the buildings was unsuitable for a tropical climate, in fact for any climate. Most of the factories had corrugated iron-roofs which absorb the heat and re-radiate it into the factory. This was such an important subject that they considered the formation of a high powered Committee of experts to study and control building construction, air-conditioning, lighting, etc., for factories an urgent necessity. He realised that under present conditions in India industries could not afford air-conditioning. Textile mills for example essentially need air-conditioning. This point has also been discussed by the post-war planning Committee (Textiles) Government of India in their last report. It was still a common belief amongst some of the textile factory owners that higher temperature and humidities reduced the warp breakages and increased the rate of production. The employers had yet to be educated to the fact that the physiological advantages that would result from air-conditioning would in the long run result in greater efficiency and output and would more than counter-balance the loss caused by higher breakages in yarn.

He pointed out that another important aspect of industrial health to which no serious attention has been paid was factory lighting. It was not realised that better lighting in a factory would result in greater efficiency leading to lower costs. A few of the factories visited however were more progressive and were launching schemes for fluorescent lighting.

The welfare activities in a good number of factories were either primitive or absent. Chilled water was not provided for thirsty workers. Washing arrangements were very inadequate. Where there were canteens the cooking and serving arrangements were dirty and the diet ill-balanced. Dr. Bedford considered that it would be in the interest and to the advantage of factory owners to subsidise canteens to enable them to serve balanced meals.

More attention could also be paid towards alleviating the postural strain in some industries. In industries where women were employed this was of particular importance.

He stated that there seemed to be a callous disregard among employers in general for industrial health hazards. Even acid burns and chrome ulcers were considered commonplace. There was practically no industry where dust was controlled. In potteries, metal grinding processes, cement mixing rooms, glass factories, sand-blasting operations, the conditions of dust control were all equally unsatisfactory. In a good number of these cases the dust contained a high proportion of silica. Some industries which tried to reduce the dust hazard by local exhausts had their ducts and hoods connected wrongly.

Dr. Bedford considered that the factory inspection system should also be tightened up. Though legislation provided adequate safe-guards this was only on paper and ' precious little ' was done.

After reviewing the existing conditions Dr. Bedford then discussed the fields in which research should be undertaken. These are referred to below :—

(a) *Thermal environment.*—This required fundamental research, as the radiant temperature was a very important factor in the worker's environment, particularly in the tropics. Researches on allied subjects like economic methods of air-conditioning, would not only be of academic interest but also interest the Indian industrialist when the findings of these investigations were extended from the laboratory to the factory. Dr. Bedford considered that a liaison between this proposed research in India and his work on the subject in England and the M.R.C. station in Singapore on the same subject should be maintained and that such liaison would be helpful in supplementing the work done in each sphere and in the training of specialists in this field.

(b) Fact finding surveys should be undertaken with a view to give a true picture of industrial health hazards and health conditions. The following were suggested :—

(1) Surveys of lighting in representative factories and correlating the results with efficiency of the workers as revealed by spoilt work or output.

(2) Surveys to correlate sickness absenteeism and accident frequency —the records for which are practically non-existent—with thermal and meteorological findings.

(3) Surveys to measure the incidence of silicosis, tuberculosis and poisoning by toxic solvents.

(c) Researches on such problems as the optimum hours of work, the arrangement of the working hours, increasing efficiency by greater mechanisation and vocational selection would give us useful results.

The members of the Committee then recounted their own experiences on some of the points mentioned by Dr. Bedford. In replying to a query from Professor Subrahmanyan Dr. Bedford explained the administrative machinery in the United Kingdom, regarding building research and factory construction. It was pointed out that the Board of Scientific and Industrial Research in India was going into the first named problem and the Committee was of the opinion that a liaison should be maintained between this Committee and the Board of Scientific and Industrial Research.

After some discussion the Committee recorded a recommendation that the researches on thermal environment, the fact finding surveys, and other problems such as optimum hours of work suggested by Dr. Bedrord should be started as soon as possible.

Dr. Bedford had stressed the necessity of improving the factory inspection system in India and when this point was discussed Mr. Peace stated that a scheme for training factory inspectors was already under way.

Dr. Bedford was of the opinion that in launching research schemes the first thing was to select suitable research workers. The terms of employment

should be such as would offer chances of permanent employment to those who proved satisfactory. It was pointed out here that the I.R.F.A. had no laboratories of their own. Dr. Bedford stated that this should not be considered as a handicap as the M.R.C. usually carried out their researches in various parts of the country and in most cases not in their own laboratories.

II. The report of the Sub-Committee on sickness absenteeism was next discussed. The report was adopted with two amendments and is attached to the report.

III. Dr. Orkney explained to the members the conditions under which the enquiry into the health of women workers working underground in mines could not be continued satisfactorily. In view of the fact that the women workers are no longer permitted to work underground the Committee did not consider the continuation of the enquiry necessary.

IV. A proposal from Dr. M. Ahmad for an enquiry into sickness absenteeism at the Bata Shoe Factory with special reference to accidents was then considered. It was unanimously decided to recommend this enquiry to the Scientific Advisory Board for approval for obtaining the necessary sanction of the Governing Body for the funds required.

V. Section 4 of the minutes of the 3rd meeting of the Medical Research Liaison Committee for India held on the 18th March 1946 at the India Office, London, was read and noted by the Committee.

VI. Lt.-Col. Lakshmanan initiated a discussion on industrial health surveys and suggested that the surveys should be started as soon as possible. The Committee agreed that such surveys were both necessary and urgent. An important item of equipment for a survey is an x-ray unit. The modern method, (which is both rapid and cheap) of roentgenological chest examinations in a health survey, is with the aid of a 35 mm. miniature x-ray film. At present the I.R.F.A. have no such equipment and the Committee, therefore, recommended that the I.R.F.A. should purchase such equipment, and keep it at a Central place like the All-India Institute of Hygiene & Public Health, Calcutta.

Dr. Bedford in this connection stated that the question was not whether to have one but 'how many' of such units.

REPORT OF THE SUB-COMMITTEE APPOINTED TO EXAMINE THE POSITION REGARDING MAINTENANCE OF SICKNESS ABSENTEEISM RECORDS IN INDIAN INDUSTRIES.

MEMBERS.

1. Lt.-Col. C. K. Lakshmanan.
2. S. C. Joshi, Esq.
3. Dr. M. N. Rao.

INTRODUCTION.

The Industrial Health Advisory Committee of the I.R.F.A. at its meeting held on 5-11-45 discussed in detail the question regarding the maintenance of records of health and sickness among factory workers. The general opinion of the Committee was that under present methods and with the present staff employed accurate data which could be used by a research worker was not available.....A Sub-Committee was appointed to examine the present position with a view to see " whether the introduction of legislation to enforce the maintenance of adequate records is a practical proposal ".

The limited experience of the Sub-Committee has been in conformity with the opinion expressed by the Committee. In a recent rapid industrial survey of thirty-four establishments it was found that twenty of them do pre-employment examinations of workers but that only two did any re-examinations with a view to follow up the health of the workers. Following up the absenteeism records with a view to analyse the health record and reduce absenteeism was not done by any of them. It would appear from this that sickness absenteeism records are maintained in very few industries.

The value of maintaining such records does not seem to be realised by the factory owners in Indian industries. In discussing the question, therefore, certain fundamental questions have to be answered. What exactly is the type of statistics which should be collected ? In what forms are the data to be collected ? Is the mechanism or personal organisation available in Indian industries adequate for collection ? What factories could be well advised to collect the information ? If legislation is suggested how best is it to be done ? The answers to these fundamental questions are attempted below.

SICKNESS ABSENTEEISM.

Sickness absenteeism is the study of time lost in industry due to illness of workers. This study is useful to the individual, in that he is in the know of his illness record and consequently he can maintain a better health status. It is useful to the employer, in that the healthier employee gives better out-turn and consequently more profits. It is useful to the community in the sense that an epidemiological assay can be made of the diseases in the community and a better control of disease maintained.

The illness of the worker is to some extent different from the illness of the other members of the population. The worker is exposed to three types of illness ; firstly, to the general community health hazards like any other member in his community. Industrialisation leads to concentration of population and

he is, therefore, exposed to the second type of illness—the illness attributable to over-crowding. That group of hazards peculiar to the type of industry he is working, forms the third group of disease the worker is exposed to.

The incidence of diseases under these three categories would obviously be different in different industries and in different countries. It is imperative therefore that whatever method of collecting sickness statistics is suggested it should be a standard one and should be such as to make them all comparable.

The usual methods of assessment.

The usual methods that could be adopted to assess sickness are as follows:

1. Analysing the out-patient statistics of the clinic in the industry.
2. Analysing the in-patient statistics of the hospital.
3. Analysing the mortality statistics of the industrial workers.

The out-patient statistics may or may not represent the correct morbidity figures of the entire industry. It has been our experience that in a small proportion of the industries the worker does not avail himself of the available medical facilities either because he does not believe in the efficacy of the doctor or the system of medicine he practises. Moreover the reliability of out-patient statistics of any hospital depends on a number of factors. The chief of them are the type of medical man diagnosing the case, the time and laboratory facilities at his disposal to diagnose each case, the knowledge of the past history of the case, and to a fair extent the physician's knowledge of industrial processes. Hence we are of opinion that morbidity evaluation from out-patient statistics may not be of great accuracy.

On the other extreme are mortality statistics. These can not offer a true picture of sickness as there are many diseases which incapacitate but are seldom fatal.

The in-patient statistics of an industrial hospital though giving valuable information regarding the common types of illnesses are only representative of a biased sample. The sick patients in the hospital do not represent the true cross section of the sick workers in the industry.

The only reliable method of assessing sickness in an industry would be to go into the deatils of the cause of each absence and if it is found to be due to sickness, to go into the details of illness.

The ideal method.

By going into such details of the cause of each absence the various causes of each absence could be classified as:—

(i) Social reasons. (ii) Non-industrial sickness. (iii) Non-industrial accidents. (iv) Industrial accidents. (v) Industrial illness.

Due to lack of available Indian data, American experience is quoted here. Ordinarily it is roughly estimated, from data from various industrial groups, that approximately 90% of the absenteeism experienced by most organisations is due to non-industrial accidents and illness, illness making up the bulk of the cause of absenteeism. The other 10% is due to industrial accidents and industrial illness, with the industrial accident proportion predominating. One of the large scale inquiries in the U.S.A. showed that non-industrial injuries and

illness accounted for 8·85 days of absence per employee per year, industrial injuries accounted for 0·59 days and occupational disease for 0·01 days. From experience in India and the U.S.A. it is felt that absenteeism due to social reasons and non-industrial illness would be more and that due to non-industrial accidents less in India than in United States. It is obvious however that an ideal sickness absenteeism record should take into consideration for both absolute and relative appraisal, all the other causes of absenteeism also.

The complete sickness absenteeism record provides facilities for further analysis. The American method has been to analyse the data as :—

(i) The disability rate : the average number of days lost per year per worker.

(ii) The severity rate : the average number of days lost per absence and

(iii) The frequency rate : the average annual number of absences per 1,000 workers. Knowing any two of the above, third can be easily computed.

The usefulness of analysing the sickness absenteeism records in the above method could be better understood from an example. (See *Appendix I*).

Rao and Ghosh while studying sickness absenteeism in a private industry during the war found that the severity rate was gradually coming down with the progress of war, though the disability and the frequency rate were on the rise. It is clear therefore that the worker is losing more time in the industry and he is absent more frequently but that each attack of illness is incapacitating him for lesser and lesser number of days ; for example in January 1941 each illness was incapacitating him for 6·3 days whereas in January 1944 for only 2·5 days. It is obvious that such a state of affairs is detrimental not only to the industry but also to the community where the worker is living. Each illness whether a cold or an intestinal upset is a source of infection in the factory and the community. Hence for the same loss of time, frequent short illnesses, as made out by this method of analysis are a worse source of ill health in the community and factory than a single illness of long duration for the same period. Any study of sickness absenteeism, therefore, should include further analysis as suggested above.

The recommended schedule.

Any method suggested, therefore, should attempt to reach this ideal. More than simple designing a schedule is the " notification, certification and verification " of the cause of absence.

Presuming that there is an industrial health set up which has all the facilities, certain basic fundamental issues have to be considered.

(i) The type of workers : As far as sickness absenteeism records are concerned there is no use collecting any statistics of the temporary worker. Just as in the case of health insurance schemes, only those workers that are permanent or are likely to be permanent are to be taken into consideration, as the records are likely to be more complete in these cases. But if any intensive study is contemplated in a part of the factory the records have to be maintained for every worker and provision made for the temporary and badli workers made during the final analysis.

(ii) Age distribution and sex distribution effect markedly the rate of sickness absenteeism in a working place. For example a factory employing older

group of workers, or more women than another factory employing the same number of workers, is bound to have a greater sickness absenteeism rate simply because women and old men get ill more easily than the others.

(iii) The type and amount of medical facilities available in the factory or the community: It is quite likely that the out-patient diagnosis by a qualified medical man of a worker who has a " Pyrexia of unknown origin " is malaria or influenza when he has a paratyphoid fever. Or again the visiting physician's diagnosis may be rheumatic fever when the laboratory diagnosis of the same case might be an atypical case of malaria. Thus in analysing the total sickness absenteeism of a factory or comparing the sickness absenteeism of one with another factory, the type of the physician, the facilities at his disposal, etc. have to be taken into consideration. Hence the overall medical experience in considering the sickness statistics can be at best on general lines.

(iv) Classification of illness: Instead of entering the individual illness, British and other groups of workers favour the entry in a pre-arranged type of classification. But it has to be realised that the classification depends entirely on the type of diseases prevalent in the community. For example, industrial neurosis may be the main cause of sickness in a factory in New England and gastrointestinal disorders the main cause of sickness in Bengal. Malaria or another type of illness common in Bengal might not be common in South India. Hence in the absence of any exact knowledge of the types of illnesses prevalent in India, it is much better to keep the data in such a way as to make it useful for anaylsis at a future date. It is, therefore, suggested that in the present schedule the diseases be recorded as such, without any attempt at analysis.

(v) The record would be best maintained in individual schedules. Instead of anticipating completely the future trend of results and collecting group data daily in the factory it would be much better to start individual cards from which groups of data of any permutation and combination could be done in the end to find out the incidence of ' accident proneness ' or again the analysis to gauge the distribution of multiple attacks of disabling sickness might lead to some interesting results.

Hence it was considered necessary to have individual records. The suggested type of record is given as *Appendix II*.

The only possible criticism for this type of record is that it does not lead to easy statistical analysis especially when large numbers have to be analysed. The answer for the criticism is that our knowledge on the subject is so meagre at present that we cannot think of designing a schedule to be easily converted into a punch card for group analysis. Moreover, the card should be so simple that a social worker should not find difficulty in filling up the schedule and so thorough, as to be amenable to complete analysis.

THE TYPE OF INDUSTRY THAT CAN AFFORD.

The recommendation of the size of the industry which should be made to maintain thorough sickness absenteeism records is not altogether easy.

The smaller industry is economically on an unstable basis and cannot possibly afford to maintain a good record system, unless on a co-operative basis. At the same time it may also be noted that in India the large majority of industries are of the smaller type. The following evidence in this connection is interesting:—

Due to lack of available data on all establishments where labour is employed, that in factories is taken as a sample though not althogether representative. Even there, the factory data in Bengal is taken as sample to represent the whole of India. Hence while considering the table below these limitations have to be borne in mind.

Distribution of Returns by size of Factories (Perennial & seasonal) in a large Indian Province in 1944.

Employee size class.	Percentage of returns.	Cumulative totals. This series.	Cumulative totals. In U.S. for comparison.	Percentage of total employment (by computation).	Cumulative totals. This series.	Cumulative totals. In U.S. for comparison.
Less than 20	13·4	..	..	0·5	..	..
21–50	36·6	50·0	..	3·4	3·9	..
51–100	20·9	70·9	..	4·1	8·0	..
101–500	17·8	88·7	63·0	11·7	19·7	13
501–1000	2·6	91·3	80	5·2	24·9	26
1001–5000	8·0	99·3	97	63·2	88·1	61
More than 5000	0·7	100·0	..	11·9	100·0	..

The important points that are obvious from the table are as follows:—

1. The small sized industry is very common in India, nearly 70% of the factories employ less than 100 workers, and more than 90% factories employ less than 1,000 workers.

2. In the United States also though the mode is the small size factory, it is not so small as in India. Nearly 40% of the factories employ labour of more than 500.

3. When the data are computed in a different fashion, however, the results are even more interesting. Only a quarter of the total industrial population is employed in factories. (India 25%, U.S.A. 26%) with 1,000 employees, and under i.e. the large majority of workers are employed in the larger establishments.

From a public health point of view, therefore, any measures taken for keeping health records in general (e.g. sickness absenteeism records) are best taken in large establishments so that a majority of workers are benefited.

The Sub-Committee considered what should be the minimum size of a large plant which should be made to maintain the sickness absenteeism records and it came to a conclusion that such of those establishments employing 2,000 labour or over must be made to keep proper sickness absenteeism records.

The large majority of such factories have already an industrial medical set up which can easily be turned to the suggested scheme of maintenance of records.

The type of personnel required.

Present knowledge of absenteeism in Indian workers is poor; it is poorer about sickness absenteeism. To appraise the variable proportion of sickness to other causes of absences it is considered that a medical social worker of the type of the industrial nurse or the health visitor should assist the industrial physicians.

Absenteeism may be due to indirect causes at home, e.g. transportation difficulties from home to the place of work, housing problems, difficulties in conducting shopping and personal business, improper arrangement at home to look after babies in arms or toddlers etc.

It might be due to certain maladjustments in the factory itself. For example, uninteresting work or a work which easily induces indifference or boredom or working hours which do not dovetail into the personal habits for the worker; in short any industrial pyschological problem may be the basis of an absentee worker.

It may also be that in some cases the worker stays away at home simply because he is entitled to do so with full pay.

The worker might be absent for private reasons or he might be ill with venereal disease and still not be absent, being afraid of victimisation by the employer. In all these instances the social worker can be of immense use to the industrial physican in the correct appraisal of sickness absenteeism.

The necessity for legislation.

Very little work on related problems has so far been done in India. The large majority of the investigations on absenteeism in general and sickness absenteeism in particular in foreign countries have been done with the help of data already collected by the establishments themselves. In Britain, the excellent work in the Welsh coal mines which formed the contents of a memoir by Bedford and co-workers was based on the absenteeism statistics maintained by the personnel department of a coal mine. The first doctorate thesis in industrial health from Harvard School of Public Health, was based on the sickness records of a public utility concern of Boston.

In all such investigations, the employers were educated about the necessity of maintaining good records for conservation of labour, and the investigations were conducted on the presumption that the records were authentic. But such circumstances do not always prevail in Indian factories. The experience of the factory inspection system in India shows that the employer does not always maintain good individual records due to ignorance of its benefits and even if the State compels him to send returns, he is reluctant to do so.

Two main points, therefore, emerge from this. Firstly, for the records to be of any use, they must be reliable. Secondly, for them to be reliable, better co-operation would be obtained by educating the employer than rather compelling him by legislation.

Present legal position in India.

The only legislation in force in British India for the collection of statistics is the Industrial Statistics Act, 1942. Section 3 of the Act provides that the Provincial Governments may direct that statistics shall be collected relating to the matters specified therein. Such matters include *inter alia*, under item (ii) of Sub-section (1), " attendance ". That in its turn, may include absenteeism for any reason, such as sickness absenteeism. It is not, however, quite clear whether the item includes " absenteeism on account of sickness ". If, according to authoritative opinion, that category is included, the work of maintaining statistics for sickness absenteeism would be easy. The Provincial Governments may, by notification in the official Gazette, direct the maintenance of statistics on the point in regard to specified industries. If, however, authoritative opinion expressed any doubt on the point or thinks that sickness absenteeism is not included under that item in Section 3 of the Act, it will be necessary to amend the Act, so that the employer is required by law to maintain statistics of sickness absenteeism in the prescribed manner. It may, however, be stated that the act itself has not been put into active operation, although it is now over four years since it was passed.

Conclusion.

At present very few industries in India maintain sickness absenteeism records. In the interests of the health of the industrial workers it is essential that correct sickness absenteeism records are maintained. The records are essentially maintained by the industry. With the help of their returns the State can assay and advise industries about their health problems. A schedule for the maintenance of records is suggested (*Appendix II*).

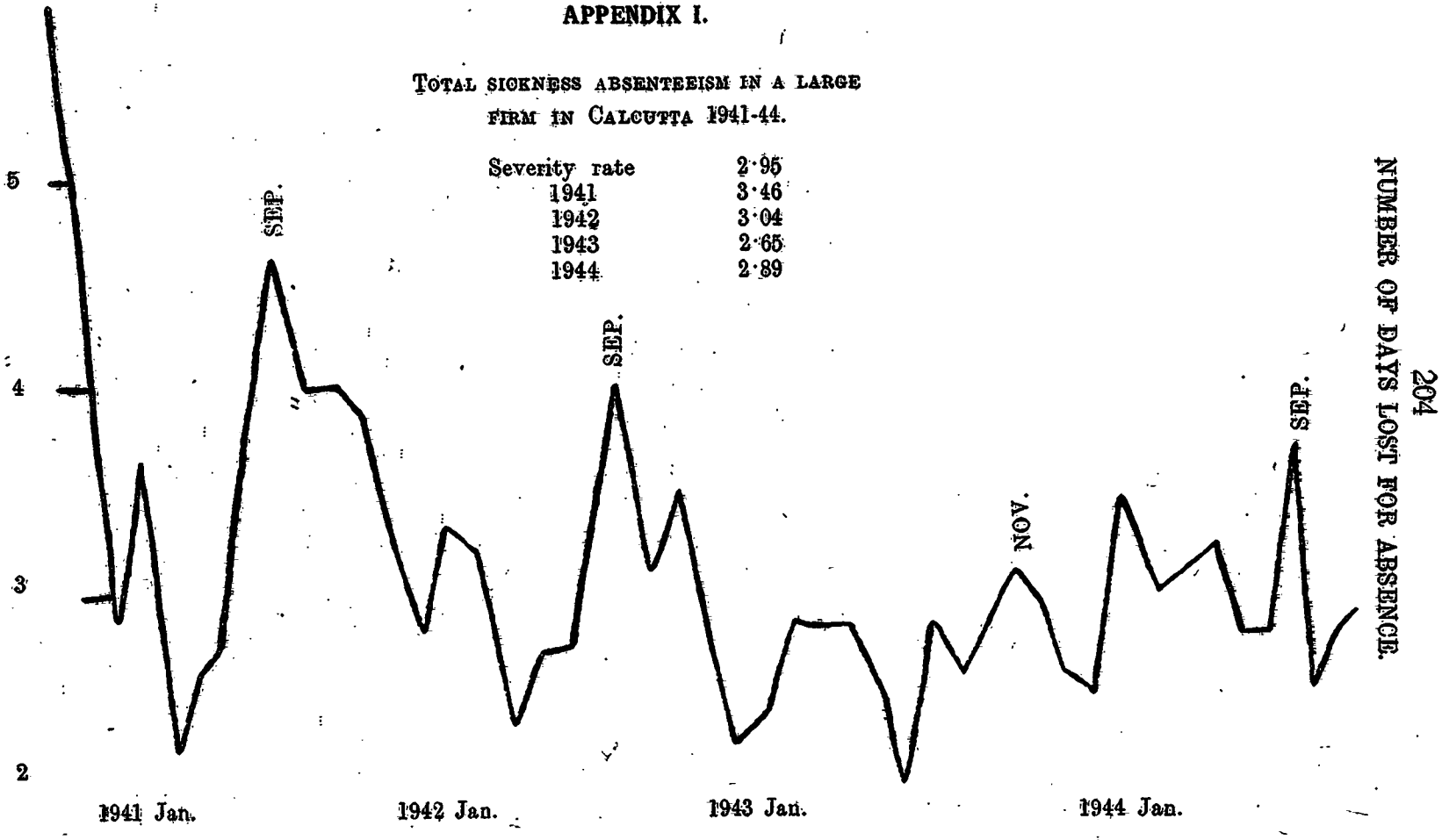
Whether the State should force the industries by legislation to maintain these records is the point in question. For example, if legislation were to be passed in Bengal all the 96 industries now in Bengal employing more than 2,000 workers would have to maintain sickness absenteeism records. The large majority of them are likely to have a skeleton medical organisation. At present there is no way of enforcing the rest to have even a skeleton medical organisation. Though total absenteeism in the factory could be elicited from the pay rolls and personnel department, to record sickness absenteeism essentially requires the presence in the factory of a qualified doctor. Without the doctor, the sickness absenteeism returns would be all uncertified sickness which serves practically no purpose. Hence unless and until there is a provision by law to compel industries to have an industrial physician on their staff no useful purpose will be served by legislation which enforces the maintenance of sickness absenteeism records.

The social insurance scheme which is expected shortly to come into force provides for a general medical set up to record, treat and follow up illness—in fact theoretically ideal conditions to maintain good sickness absenteeism records. Hence we are of opinion that any legislation at present would be premature. While awaiting the comprehensive social insurance scheme, the Sub-Committee is of opinion that large scale industries should be encouraged to maintain good sickness absenteeism records.

APPENDIX I.

TOTAL SICKNESS ABSENTEEISM IN A LARGE FIRM IN CALCUTTA 1941-44.

Severity rate	2·95
1941	3·46
1942	3·04
1943	2·65
1944	2·89



APPENDIX II.

All-India Institute of Hygiene & Public Health, Calcutta.
Department of Physiological Hygiene.

ABSENTEEISM RECORD.

Instructions for filling.

1. Cross all the holidays (X this way) i.e. those days when
   (i) either the plant is closed,
   (ii) the worker gets his weekly day off,
   (iii) the management legitimately permits the worker to be absent in lieu of overtime etc.

   Remember the worker is getting full pay for all the days marked (X).

2. Mark off by a wavy line (~) those days he is either not in employment of this company or on strike or discharged temporarily by the company. Also the days before you commenced this enquiry or the day terminated this enquiry. In each case, however, write why the wavy line is drawn.

3. Each day (or square in the schedule) can be divided into a forenoon and afternoon whenever necessary, by a horizontal line.

4. The first day (or only the afternoon as the case may be) he is absent mark *A*. The letter A on any one day means that from that day onwards he is absent. If he is absent only for that day write the reason of his absence in that square only. Ask him if he is staying in a house provided by the factory. If so circle the letter A. If not do not circle.

5. If he is absent for more than one day *continue drawing dashes each day* and the last day (or half day) of each absence is to be marked by one of the following letters :—

   C.S.—Certified sickness.

   U.S.—Uncertified sickness.

   C.A.—Certified accident.

   U.A.—Uncertified accident.

   H.W.—Home work.

6. After thus marking the first and last days of the absence write in the intermediate days the cause of absence.

   (i) *If you do not mention the cause in between, the records will be very faulty.* It shows that the worker is absent for two days only ; the first and last day.

   (ii) In case of certified sickness and accident write the doctors' diagnosis.

   (iii) In case of uncertified sickness and accidents write what the worker says. In filling use the following :—

   G.I. Gastro-intestinal diseases, e.g. diarrhoea, dysentery, stomach ache, etc.—remember typhoid fever is a gastrointestinal disease.

   I.D. Other common infectious diseases. Include in this smallpox, chickenpox, mumps and meningitis.

F. Include in this all unspecified fevers including malaria.

R.D. Respiratory diseases including common cold, influenza and pneumonia.

Use these letters only in the absence of medical diagnosis.

7. Do not fill up more than hundred cards from the same department.

8. If the enquiry is being done more thoroughly—it may also be noted whenever the worker is ill but still works. In such cases instead of starting with A as in the case of absence start with P (meaning he is present) and continue as before.

Your signature and designation please :

Please turn over.

NAME OF THE INDUSTRIAL ESTABLISHMENT : *ADDRESS :*

Year	Name	Dept.	W.D.	D.A.	N.A.	A.N.D.	A.N.E.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Jan.																															
Feb.																															
Mar.																															
April																															
May																															
June																															
July																															
Aug.																															
Sep.																															
Oct.																															
Nov.																															
Dec.																															
Totals																															
Analysis of sickness																															

History. Personal.
Occupational

Age
Past

Years.
Date Entered.

Sex

Date left.

Duration.

Name of the Establishment.

Occupation.

Present :

Details of present occupation :
Shifts A.M. to and P.M. to During

W.D. = Working days.
D.A. = Days absent by sickness.
N.A. = No. of absences by sickness.
A.N.D. = Average daily No. of workers in dept.
A.N.E. = Average daily No. of workers in the whole estt.

MINUTES OF THE THIRD MEETING OF THE INDUSTRIAL HEALTH ADVISORY COMMITTEE HELD ON THE 11th DECEMBER, 1946 AT 2-30 P.M. IN THE HAFFKINE INSTITUTE, BOMBAY.

MEMBERS PRESENT.

Lieut-Colonel C. K. Lakshmanan, I.M.S., Director, All-India Institute of Hygiene & Public Health, Calcutta. (*Chairman*).

Dr. B. C. Das Gupta, Executive Health Officer, Bombay Municipality, Bombay.

Mr. S. P. Jain, Non-Medical Statistician, Office of the Director General, Indian Medical Service, New Delhi.

Major E. L. Jones, I.M.S., Deputy Director General (Social Insurance), Indian Medical Service, New Delhi.

Mr. S. C. Joshi, M.A., LL.B., Chief Labour Commissioner to the Government of India, Department of Labour, New Delhi.

Mr. N. S. Mankiker, B.Sc., B.E., Deputy Chief Adiviser on Factories, Government of India, Department of Labour, New Delhi.

Major J. R. Sen, I.M.S., Office of the Public Health Commissioner with the Government of India, New Delhi.

Prof. K. Subrahmanyan, B.E., C.E., S.M., M.I.E., A.M.I.C.E., Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. M. N. Rao, M.B.B.S., M.P.H., Dr. P.H., Assistant Professor of Physiological Hygiene, All-India Institute of Hygiene & Public Health, Calcutta. (*Secy.*)

Rao Bahadur Dr. M. G. Kini, Dr. J. M. Orkney and Dr. J. N. Ray were unable to attend the meeting.

BY INVITATION.

Professor William Adiseshiah, St. Stephen's College, Delhi.

Rai Bahadur Dr. A. C. Banerjea, C.I.E., Director of Public Health, United Provinces, Lucknow.

Colonel E. Cotter, C.I.E., V.H.S., I.M.S., Public Health Commissioner with the Government of India, and Secretary, Indian Research Fund Association, New Delhi.

Sir Wilfred Garret, Chief Adviser on Factories, Department of Labour, New Delhi.

Mr. T. Gonsalves, B.A., LL.B., F.R.E.S., Assistant Secretary, Indian Research Fund Association, New Delhi.

Lieut.-General R. Hay, C.I.E., K.H.P., I.M.S., Director-General, Indian Medical service, New Delhi.

Mr. W. H. Kirby, C.I.E., Rationing Adviser to the Government of India, Department of Food, New Delhi.

Rao Bahadur Dr. K. C. K. E. Raja, O.B.E., Officer on Special Duty (Planning & Development), Office of the Director-General, Indian Medical Service, New Delhi.

1. TO CONSIDER THE RECOMMENDATIONS MADE BY DR. THOMAS BEDFORD IN THE FIELD OF INDUSTRIAL HYGIENE IN INDIA AND TO FORMULATE PLANS FOR THE ADVANCEMENT OF RESEARCH ON THE SUBJECT.

The Chairman reviewed briefly the recommendations made in Dr. Bedford's report. He laid stress on the following points :—

(a) The formation of a sub-committee to study factory construction, ventilation and sanitation ;

(b) The improvement of the present factory inspection system ;

(c) The formulation of plans for future research in industrial health ;

and (d) The setting up of a medical inspectorate for factories.

In regard to (a) :—The Committee felt that the time was not yet ripe for the formation of a sub-committee consisting of medical men and engineers to study this important subject. The sub-committee will not be in a position to make concrete recommendations regarding the construction of factories until standards for ventilation, light and other environmental factors are determined. Fact-finding surveys and investigations will have to be carried out to obtain the data necessary for laying down these standards.

In regard to (b) :—Mr. Mankiker explained that steps are being taken to tighten up factory inspection. He made special reference to the training of junior factory inspectors, issue of technical bulletins dealing with factory inspection and the modification of the Factories Act based on recommendations made at a recent conference of Factory Inspectors from provinces and states.

In regard to the training of factory inspectors, the committee were of opinion that more importance should be given to the practical aspects of industrial health and medicine. The Chairman in this connection stated that the All-India Institute of Hygiene and Public Health, Calcutta, would be glad to give all possible assistance in the training of factory inspectors.

In regard to (c) :—The Chairman pointed out that practically no research in the field of industrial health has so far been conducted. There are, however, many urgent problems which need investigation. So far, only three proposals for research on this subject have been received. This being the position, industrial health research would not proceed apace if it was left entirely to individual workers. The Chairman felt that a start in the right direction would be the setting up of an industrial health research unit which could take up urgent problems. The workers trained by this unit would blaze the trail of industrial research in the Universities.

Colonel Cotter pointed out that historically there was much to be said for the establishment of such a pioneer unit. McCarrison's nutrition research under the Indian Research Fund Association had started on these lines. The research workers who would gain experience in such a unit would be available for starting other units in the medical colleges and university departments. The All-India Institute of Hygiene and Public Health, Calcutta, has a Department of Industrial Hygiene but the routine duties of the staff did not permit of their being able to pursue research on an effective scale. He, therefore, thought the Chairman's proposal both sound and reasonable.

After discussion the Committee recommended that a unit consisting of a physiologist, psychologist, chemist, medical officer and an engineer be set

up at the All-India Institute of Hygiene and Public Health, Calcutta. This group of workers will no doubt have to be trained in their respective fields before they can undertake research. A detailed estimate of the cost of the unit has not yet been worked but it is estimated that it will be in the region of Rs. 30,000.

The Committee decided to discuss (d) under Agenda Item No. 3.

The Chairman invited further comments from members of the Committee in regard to Dr. Bedford's recommendations. It was suggested that a sub-committee be appointed to collect statistical data at present available regarding sickness absenteeism and accidents. The Committee was informed that the Industrial Health Insurance Scheme was likely to come into force from the 1st January, 1948, and that in about six months from that date, reliable statistical data on sickness absenteeism would be available. The Committee therefore did not consider it necessary to appoint a sub-committee for this purpose.

The Committee on being informed that a team of workers are engaged on industrial psychology at the Tata Works, Jamshedpur, recommended that a member of this team should be invited to attend future meetings of the Industrial Health Advisory Committee.

2. To consider proposals for new enquiries.

(i) Enquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Professor K. Subrahmanyan, Professor of Sanitary Engineering, All-India Institute of Hygiene and Public Health, Calcutta.

The Committee recommended a grant for this enquiry. In recommending this grant, the Committee suggested that instead of carrying out investigations in one or two of a large number of different types of factories, it might be better to limit the investigations to a larger number of factories of the same type.

(ii) Enquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under Dr. M. Ahmad, Chief Medical Officer, Batanagar.

A grant was recommended for this enquiry. The Chairman informed the Committee that it was likely that Dr. Ahmad will not be available at Batanagar to carry out the enquiry but if this happened his successor would in collaboration with the Industrial Hygiene Section of the All-India Institute of Hygiene and Public Health, Calcutta, be able to conduct the enquiry.

(iii) Enquiry on fatigue and efficiency in paper and textile industries under Professor Kali Prasad at the Laboratories of Experimental Psychology, Lucknow University, Lucknow.

A grant was recommended for this enquiry. The Committee recorded the opinion that the scope of work covered by the enquiry was too wide and should therefore be restricted to one type of factory, either paper or textile, where full co-operation of the factory authorities will be available. The Committee felt that Professor Kali Prasad might while conducting the enquiry maintain contact with Professor William Adiseshiah of the Delhi University, who also has considerable experience in the field of industrial psychology.

3. To consider a note from Lieut.-Colonel C. K. Lakshmanan, Director, All-India Institute of Hygiene and Public Health, Calcutta, on the desirability of instituting a Medical Inspectorate of Mines and Factories in India.

The Committee, after discussing the note adopted it with certain minor corrections. The corrected note is printed as *Appendix I* to this report.

Sir Wilfred Garrett stressed the importance of the Medical Inspector working as a member of a team of the Factory Inspectorate.

4. To consider the purchase of mass chest radiographic equipment.

The Committee discussed the subject in detail and recommended that a sum of Rs. 1,15,000 be provided for the purchase of Mass Chest Radiographic equipment. They further recommended that the Government of India be approached for a special grant to cover the cost of this important and necessary equipment.

5. To consider a note on the propagation of knowledge connected with hazards in industry.

The Committee read with great interest the documentation presented and recorded their appreciation of the work done by the Public Health Commissioner with the Government of India in preparing the abstracts on the subject and giving them wide circulation.

The Committee hoped that the publication of these abstracts will continue.

6. To note section 3(b) of the minutes of the 5th meeting of the Medical Research Liaison Committee for India held on the 10th September, 1946.

The Committee appreciated Dr. Bedford's offer of assistance to workers from India who may proceed to the United Kingdom to see industrial health work.

Sir Wilfred Garrett stated that he would also be glad to provide facilities for workers in industrial health who may proceed to the United Kingdom in future.

7. To consider section 4 of the minutes of the 5th meeting of the Medical Research Liaison Committee for India held on the 10th September, 1946.

The Committee noted the communication from Professor Lee regarding the physiological and psychological factors affecting human life under tropical conditions.

(8) Note by the Chief Adviser, Factories, Government of India, on temperature and humidity in cotton factories.

The object of the Chief Adviser, Factories, in placing the note before the Committee was to draw its attention to the necessity for authoritative medical opinion on environmental conditions—temperature, humidity and ventilation—that should be accepted as standards in Indian cotton mills. The Committee while accepting the necessity for such standards felt that until fundamental research on these subjects is carried out it would not be possible to give an authoritative medical opinion.

APPENDIX I.

Desirability of instituting a Medical Inspectorate of Factories and Mines in India.

(I) Introduction.

The object of this note is to emphasise the urgent need for the initiation of a Medical Inspectorate of Factories and Mines in India.

The present factory inspection system in India was originally adopted on the lines of the system prevailing in the U.K. In the actual operation of the system, however, some gaps were left. This has resulted not only in inadequate administration of the Factory Act but has also led to a sad neglect of the medical aspect. It cannot be too strongly emphasised that the efficiency of industry and prosperity of a nation are intimately linked with the well-being and health of its industrial population. The medical inspection and control of factories, with a view to improve the worker's health, is and ought to be thus a factor of paramount importance in any system of factory inspection. This aspect, so far neglected in India, needs to be urgently remedied. Comparative study is therefore made hereunder of the factory inspection system at present prevailing in England and in India, based on the available information. As a result of the study, it is suggested that a Medical Inspectorate of Factories and Mines be urgently installed both at the centre and the provinces to supplement this hitherto neglected aspect of factory inspection.

(II) The present organisation of the Factory Department in the U.K.

The department entrusted with the inspection of factories and the administration of Factory Act is known as the Factory Department. It would appear that mines do not at present come under the same Department, but have a separate inspectorate of their own under the Ministry of Fuel and Power. The Controlling head of the department is the Secretary of State for Home Affairs. The affairs of the Factory Department occupy, however, a tiny portion of his time and are mainly under one of the Under Secretaries of State for Home Affairs who again delegates these powers to an Assistant Under Secretary of State, who is the head of the Industrial Division. In 1940 the control of the Factory Department was transferred from the Home Office to the Ministry of Labour and National Service, where its functions are guided by the Secretary of State who is the head of the department concerned with safety, health and welfare of persons employed generally outside of mines. The head of the Factory Department is the Chief Inspector of Factories, assisted by four Deputy Chief Inspectors, all being stationed in the Factory Department. The work of the Deputy Chief Inspectors is specialised and each is in charge of specific matters such as employment and hours of work, engineering side of industries and rules and regulations. The various sections, however, though demarcated administratively, always work as a team.

For administrative purposes, Britain is divided into twelve divisions of inspection each placed under a Superintending Inspector. There is a Deputy Superintending Inspector in each division but that office is usually occupied by a woman Inspector when the Superintending Inspector is a man and *vice versa*. The twelve divisions of inspection are further divided into ninetytwo districts, each under one District Factory Inspector assisted by two other inspectors.

In the more important districts, there may be three other inspectors; in less important ones, there may be only one or none.

The Minister decides with the approval of the treasury, the number of inspectors and their salary. Their numerical strength in 1945 was 440, but these now include about 30 special advisers on canteens and personnel management. Vacancies for new appointment arise in the lowest grade only and at a minimum salary. A newly recruited inspector is given two weeks' intensive training by the Factory Department and besides theoretical instruction, is initiated in his work by going round the factories with an experienced inspector. After sometime he may be given smaller factories to inspect. The first two years are spent on probation and confirmation follows only after a departmental examination.

The Factory Inspectorate is divided into (i) General Staff and (ii) Technical Staff both being under the control of the Chief Inspector and his deputies.

(i) *General staff*:—This includes the Chief Inspector, the Deputy Chiefs, the Superintending Inspectors, Class IA Inspectors, Class IB Inspectors and Class II Inspectors or Juniors. Inspectors from Superintendents downwards are subject to rotation. They are transferred from district to district every 2-3 years, so that 7-8 years after his appointment an inspector will have served in about three different districts, probably a maritime district, a textile district and a general district. He is then qualified for promotion to Class IB and is placed in charge of a district of lesser importance. After another 5-6 years he is transferred to a more important district and will become Class IA Inspector. He may afterwards be promoted to upper grade such as Superintendentship and so on as occasion arises.

(ii) *Technical staff*:—The technical side is at present divided into the following four branches:—

(A) The Medical,

(B) The Electrical,

(C) The Engineering and Chemical,

(D) The Textile Particulars.

The present Senior Engineering Inspector has a staff of nineteen under him. There are fourteen Medical Inspectors serving under a Senior Medical Inspector. The electrical and textile particular branches have fourteen and five Inspectors under a Senior Inspector each.

Medical Inspection and control of Factories.

For the Medical Control of factories, the Medical Inspectors are assisted by the Examining Surgeons. Under the present system the Chief Inspector or the Superintending Inspector, in cases where the Minister so directs, may appoint a sufficient number of duly qualified medical practitioners to be Examining Surgeons. These are to be paid for per case by the employers *The Examining Surgeon for any factory shall have the power at all reasonable times to inspect the general register for that factory and when he investigates the factory accidents or industrial diseases, he shall have "the like powers as an Inspector, including power to enter any room in a building to which the person killed, injured or affected has been removed".*

There are about 1,750 Examining Surgeons in Great Britain. Most of them were part-time officers and all of them were given a certain district within which they might perform their duties under the Factories Act. *They are under the direct control of the Medical Inspectorate of the Factory Department.* Besides their official duties, those, who are part-time examining surgeons, have their private practice within the district. The private practice and the official duty of the examining surgeons not only do not conflict with each other but are together conducive to good results.

The main duty of the examining surgeon is of course the medical examination of young persons for factory employment but perhaps his most important duty is the medical examination of persons employed in certain hazardous occupations where such examinations (generally monthly) are required by regulations. The examining surgeon has other official duties to perform under the Factories Act of 1937.

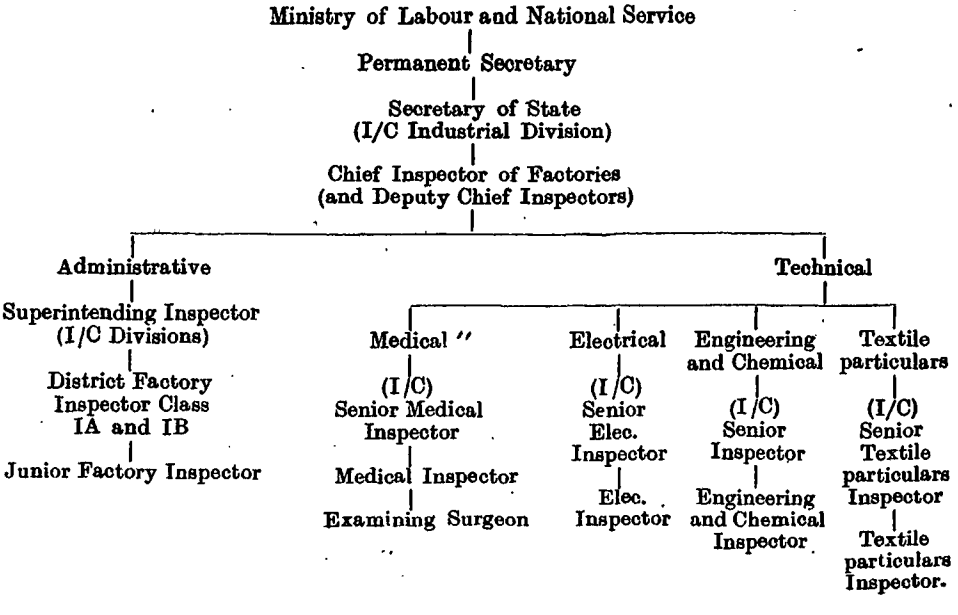
First he shall make special enquiries and examinations of employed persons as may be directed by the Secretary of State.

Secondly, he shall investigate into and report on cases of death and injury caused by fumes or other noxious substances or any other special cause specified; upon any case of death or injury validly referred to him by the factory inspector or upon any case of disease notified under the Act.

Thirdly, he shall report annually at a time and in the form prescribed by the Home Secretary as to the examinations made and other duties performed in pursuance of the Factory Act. For investigations into and the report on factory accidents or industrial diseases as well as the making of annual reports to the Home Office the examining surgeon is not paid any fees.

The Organisation is summarised below :—

Factory inspection system in England.



(III) Present position in India.

Each of the provinces in India has a Chief Factory Inspector usually an engineer, who has got a small number of assistants, mainly engineers. The Central Government has an adviser on factory problems attached to the Labour Department.

The most notable feature about this factory inspection system is that the Factory Inspectors entrusted with the inspection of factories and mines are all engineers and the counterpart of Medical Factory Inspector in the United Kingdom is conspicuous by its absence. The Factory Inspectors so recruited have necessarily no basic training in health problems and particularly problems of occupational diseases. In this connection, it is pertinent to point out that in the U.K. a departmental examination dealing with probelms of factory inspection and industrial health is considered essential before the newly recruited factory inspector is considered fit to carry out his duties and confirmed after two years. No such test has so far been applied in India.

The only apology for medical inspection and control in respect of factories and mines, if it can be called control at all, is the appointment of certifying surgeons by the provincial governments. Not all provinces have whole time certifying surgeons. The senior district medical officers of health in the provinces are by statute additional factory inspectors but the inspection is only obligatory in nature. Bengal has only one whole time officer for this purpose. It is appalling to think that Bengal with an area nearly that of the U.K. and an industrial population approaching a million mark should have just one certifying surgeon as against nearly two thousand examining surgeons in the U.K. Further the work of even this certifying surgeon or the part time medical officers is confined merely to certify the age of the worker whereas that of the Examining Surgeon in the U.K. comprises also diagnosis and reporting of occupational injuries, accidents and diseases. It may be mentioned that the position of mines in this respect is not much better. The mines inspection system is entirely under the Central Government with no medical man attached, corresponding even to the certifying surgeon of the Factory inspection system. The health organisations in established mining settlements like Coal mining—the Mines Board of Health and miner's welfare fund—have their activities limited only to the surface and not to the environmental conditions in the mine.

(IV) Suggestions.

Urgent re-organisation of the Factory Inspectorate in India is, therefore, needed. Mr. Peace told us in the last meeting that the engineering aspect of the system is being re-organised. As pointed out earlier, the re-organisation on the engineering side alone cannot afford to make up for the medical man's job.

The responsibility of reporting on certain industrial accidents and diseases rests on the Examining Surgeon in the U.K., whereas in India it is the sole responsibility of the Factory Inspector, who is only an engineer. It is obvious from the above that the medical man has as much as or even more responsibility in factory inspection. The present position of an engineer being expected to do the medical man's job in the vast field of occupational diseases and advising the factory management on general health matters appears therefore anomalous.

A Chief Medical Inspector of Factories with a skeleton staff at the centre of the country or a province on the lines of the United Kingdom would, therefore, appear to be urgently necessary.

In organising the Medical Inspectorate, an important aspect to be remembered is the relationship between the Provinces and the Centre. In other words, like the Inspectorate of Factories at present it is necessary to have a Central Advisory Unit with provincial executive units or, like the Mines Inspectorate system, centralise the entire organisation. In view of the fact, however, that the future relationship of the Centre may be with groups of provinces rather than with individual provinces, it is suggested to have at present a Central Advisory Unit as well as a unit in each of the provinces. In the initial stages of the development of the Medical Inspectorate each of these advisory units may comprise only one medical inspector with laboratory facilities. In the later stages, however, a greater number of medical inspectors and examining surgeons with wider facilities should be the target.

RABIES ADVISORY COMMITTEE.

The Committee met at 10-00 a.m. on December 10, 1946.

PRESENT.

Major-General Sir Gordon Covell. (*President*).
Lieut.-Colonel M. L. Ahuja.
Lieut.-Colonel H. W. Mulligan.
Dr. R. Sanjiva Rao.
Dr. N. Veeraraghavan.
Dr. A. G. Brooks. (*Secretary*).

Colonel E. Cotter, I.M.S., Secretary of the Scientific Advisory Board of the Indian Research Fund Association kindly attended the meeting.

The following were also present :—
Dr. C. G. Pandit.
Mr. P. Bruce White.

(1) Lieut.-Colonel Ahuja produced evidence to show that the 'Culture Vaccine' as prepared by Dr. Veeraraghavan at Coonoor was a distinct advance on present methods of preparation of anti-rabic vaccines. It was resolved that Dr. Sanjiva Rao be requested to repeat experiments with the "culture vaccine" on large batches of mice. It was also recommended that Colonel Ahuja put forward a request to the Secretary, Pasteur Association of India for funds to erect suitable animal houses at Coonoor for the housing of monkeys for rabies research experiments.

(2) The Rabies Advisory Committee recorded their appreciation of Dr. Veeraraghavan's recent work on rabies research carried out since its last meeting. In the opinion of the Committee this work represents a definite and important advance towards the development of anti-rabic vaccine free from the disabilities attached to this at present in general use.

(3) The Committee discussed at length the question of acquiring a suitable site for the promotion of anit-rabic research.

The urgent necessity for the provision of a second centre for anti-rabic research recognised at last year's meeting was further impressed on the Committee after hearing the account of the recent research work carried out at the Pasteur Institute, Coonoor. The Committee considered it imperative that the work be conformed by an independent set of workers, but regretted that no suitable centre existed at present where this work could be undertaken.

It was agreed that Kasauli offered the most favourable facilities for the setting up of a research centre, both by reason of its location and because of the presence there of a number of trained personnel possessing many years of experience of anti-rabic work acquired by them when members of the staff of the Pasteur Institute of India.

It was agreed that the Pasteur Association of India should be asked to provide funds for the acquisition of a site in Kasauli for the erection of necessary buildings or alteration to existing buildings, and that the responsibility for

providing the necessary funds for the scheme be accepted by the Government of India or the I.R.F.A.

The Director, Central Research Institute, Kasauli, undertook to put up concrete proposals on the basis of which the Pasteur Association of India could be asked to provide the necessary funds.

(4) The Committee considered in detail the proposal for a research enquiry put up by Major K. Banerjee and did not consider it suitable for recommendation to the Scientific Advisory Board.

INDIAN JOURNAL OF MEDICAL RESEARCH AND INDIAN MEDICAL RESEARCH MEMOIRS.

1. (a) *The Indian Journal of Medical Research, Volume XXXIII,* 1945.—Ony two numbers of the Journal were published, and these appeared in May and October respectively. The new style of printing with narrower margins was continued in the interests of economy. The average size of each number was 160 pages of printed and tabular matter, with four plates, representing an increase of 34 pages per number as compared with Vol. XXXII.

(b) *The Memoirs.*—No Memoir was published with Volume XXXIII as no material was received which satisfied the requirement that the work done would be likely to aid the war effort.

(c) *Cost.*—The net cost of publication of Volume XXXIII (two numbers only) was Rs. 3,391/-.

2. (a) *The Indian Journal of Medical Research, Volume XXXIV,* 1946.—This volume consisted of two numbers only which were due to appear in May and October respectively. Unfortunately there was considerable delay in their appearance, the May number being published in July and the October number in the following January. Typescripts were submitted to the publishers in good time but owing to civil disturbances, postal strikes, etc., delays were inevitable. The May issue contained 205 pages and 1 plate, while the October number contained 122 pages and 2 plates. Volume XXXIV was slightly larger than Volume XXXIII.

(b) *The Memoirs.*—As no suitable material was received, no Memoir was published with this volume.

3. *Distribution* (1945).—(Figures for 1946 will be given next year):

Indian subscribers	149	=244
Foreign subscribers	95	
Exchange list	189	=319
Free list	130	
Voucher copies to advertisers, regn, make-up, police, specimen, propaganda, etc.		20
Balance left with publishers for sale		117
Total copies printed		700

The exchange and free lists have been increased by the addition of 2 and 4 new addresses respectively. Issues to 72 foreign addresses on the distribution list were suspended owing to the war, but it has since been possible to resume issue to about half of these, and it is expected that most of the remainder will be resumed shortly. Similarly the number of publications received in exchange for the Indian Journal of Medical Research has increased, owing to receipts of journals from liberated countries.

4. *Distribution Lists.*—The existing lists were last revised in July 1938. Steps are now being taken to collect the information necessary to undertake a complete revision of these lists.

5. *Number of copies printed.*—Only 700 copies of the Journal have been printed since July 1940 instead of the previous 900. This number is considered sufficient to meet all current demands. Vols. I-VIII of the Journal are out of print.

6. *Cost of printing the Journal.*—The following statement shows the cost of production during the last five years :—

Cost of Printing.

Number	XXIX-XXX 1941	XXX 1942	XXXI 1943	XXXII 1944	XXXIII 1945
April	Rs. 2,052	2,949	May 2,633	May 2,116	May 4,037
July	Rs. 3,531	1,650	..	..	..
October	Rs. 3,413	2,413	2,486	2,752	2,777
January	Rs. 3,531	..	..	..	..
Totals	Rs. 11,527	7,012	5,199	4,868	6,814
Average pages	214	158	125	126	160

Recoveries.

April	Rs. 1,325	1,034	May 837	May 1,293	May 1,842
July	Rs. 1,325	946	..	..	..
October	Rs. 837	1,518	880	..	1,580
January	Rs. 837	..	..	..	..
Totals	Rs. 4,324	3,498	1,717	1,293	3,422

7. *Net cost of Journal and Memoirs.*—The following statement shows the net cost of the production of the Journal and Memoirs during the last five years :—

Journal.

Number	1941	1942	1943	1944	1945
April	Rs. 727	1,915	May 1,796	May 823	May 2,194
July	Rs. 2,206	704	..	..	..
October	Rs. 2,576	895	1,606	2,752	1,197
January	Rs. 1,694	..	..	..	..
Totals	Rs. 7,203	3,514	3,402	3,575	3,391

Memoirs.

No. 33	Rs. 1,565	..	..	..	..
No. 34	Rs. 1,627	..	..	..	..
Totals	Rs. 3,192	..	..	..	..
Grand Totals	Rs. 10,395	3,514	3,402	3,575	3,391

For convenience of accounting all receipts are credited to the Journal account, and the figures given for the Memoirs are the actual cost of production.

8. *Financial position for the year* 1946-47.—The budget for the year provided for Rs. 10,500/- to meet the cost of two half-yearly numbers and one quarterly number (January 1947) of the Journal and Rs. 2,000/- for any Memoir which may be published. The following is the estimated expenditure :—

Volume XXXIV, No. 1 (May 1946) No. 2 (Oct. 1946) Volume XXXV, No. 1 (Jan. 1947)	@ Rs. 3,000 each	Rs. 9,000 approx.
No Memoir was published		Nil.
	Total	Rs. 9,000

9. *General.*—The editorial policy of the Journal was revised last year on the recommendations of the Scientific Advisory Board and the Editorial Board was enlarged to include 25 referees. A note for the guidance of members of the Board was also prepared by the Editor and circulated to all concerned. The services of the members of the Board have been fully utilized in accordance with the policy adopted.

The Scientific Advisory Board has decided to recommence publication of quarterly issues of the Journal with effect from January 1947, when the fresh number of Volume XXXV is due to appear. It may, therefore, be anticipated that 4 numbers will appear during the calendar year 1947, in January, April, July and October respectively. Now that the paper shortage is no longer acute, it has been decided to revert to the old style of printing with wider margins and narrower columns.

Efforts have been made in investigating other avenues for the speedy publication of the Journal, but owing to reluctance on the part of other suitable printers to undertake new work, there is no alternative but to continue with present arrangements for the time being.

To avoid delay in handling correspondence the Senior Assistant Director of the Central Research Institute, Kasauli, has been appointed as the Assistant Editor of the Journal as from May 1946.

All the papers published in Vol. XXXIV were submitted by workers in India or were the results of investigations carried out in India. The lines of special research activity are reflected in the nature of papers received. Of the 41 articles published in this volume, 23 were on nutrition, 3 on bacteriology, 7 on biochemistry, 2 on pharmacology, 1 on haematology, 2 on rabies, 1 on snake venom, 1 on helminthology and 1 on serology.

The size of the Journal has been considerably reduced since 1944 owing partly to the new style of printing adopted, partly to a reduction in the number of papers received for publication and partly to the efforts made by contributors to reduce their papers to minimum size.

10. *Budget esimate for* 1947-48.—The following is the estimated cost during the year for 700 copies of 4 quarterly numbers of the Journal and 1 possible Memoir :—

4 quarterly issues of the Journal @ Rs. 3,500	..	Rs. 14,000
1 Memoir	..	Rs. 2,000
Total	..	Rs. 16,000

The cost of individual numbers of the Journal remaining the same the annual subscription will be reverted to Rs. 20/-.

Publications on medical research under the auspices of the Indian Research Fund Association during the year 1946.

A. *Indian Journal of Medical Research and Indian Medical Research Memoirs.*

1. AHUJA, M. L., BROOKS, A. G., VEERARAGHAVAN, N., and MENON I. G. K.—A Note on the Action of Heparin on Russell's Viper Venom (Vol. 34, No. 2).
2. BASHIR AHMAD, RAM CHAND and MANSOOR-UL-HASSAN.—Observations on the Vitamin A Content of Buffalo Butter-Fat (Ghee): Effect of the Method of Preparation, Storage and Cooking (Vol. 34, No. 1).
3. BASU, K. P., and MALAKAR, M. C.—Destruction of Vitamin B_1 of some Vegetables during Cooking and the Effect of Cooking on Free and Combined Vitamin B_1 of some Foodstuffs (Vol. 34, No. 1).
4. BASU, K. P., and NATH, H. P.—The Digestibility of certain Vegetable Oils and Fats determined by Metabolic Experiments on Human Beings (Vol. 34, No. 1).
5. BASU, K. P., and NATH, H. P.—The Rate of Absorption of different Fats and Oils (Vol. 34, No. 1).
6. BASU, K. P., and NATH, H. P.—The Effect of different Fats on Calcium Utilization in Human Beings (Vol. 34, No. 1).
7. BASU, K. P., and NATH, H. P.—Comparative Value of Butter-Fats and Vegetable Oils for Growth (Vol. 34, No. 1).
8. BHAGVAT, K.—The Effect of Hesperidin and a Factor in Bengal Gram (*Cicer arietinum*) on the Growth of Guinea-pigs (Vol. 34, No. 1).
9. BHAGVAT, K.—Toxic Effects in Guinea-pigs of Diets containing Large Proportions of *Lathyrus sativus* (Vol. 34, No. 2).
10. DAS GUPTA, B. M., and SEN GUPTA, R. L.—Serological Studies in a Case of Acute Hæmorrhagic Jaundice (Vol. 34, No. 2).
11. DE, N. K., RANGANATHAN, S., and SUNDARARAJAN, A. R.—Vitamin A and Carotene Content of Ghee (Clarified Butter) and 'Fortified' Margarine (Vol. 34, No. 1).
12. DHARMENDRA, BOSE, R., and SEN GUPTA, P. C.—The Preparation of an Antigen from the Kedrowsky's Bacillus for the Complement-fixation Test for Kala-azar (Vol. 34, No. 1).
13. DHARMENDRA, and MUKERJEE, N.—Action of Penicillin on *Mycobacterium leprœ* Muris (Vol. 34, No. 2).
14. DIKSHIT, P. K. and PATWARDHAN, V. N.—Studies in Experimental Rickets. The Changes in the Ionic Products of Calcium Phosphates and the Serum Alkaline Phosphatase with the Onset, Progress and Healing of Rickets (Vol. 34, No. 2).
15. GREVAL, S. D. S., BHATTACHARJEE, J. N., and DAS, B. C.—Complement-fixation Reaction between Serum-Protein and its Antibody for Medico-legal Purposes; and Associated Immunological Considerations (Vol. 34, No. 2).
16. GUPTA, J. C., ROY, P. K., and DUTT, A.—Pharmacological Action of an Active Constituent isolated from *Dœmia extensa* Linn. (Syn. *Pergularia extensa*). Part I (Vol. 34, No. 1).

17. GUPTA, J. C., ROY, P. K., and SEN GUPTA, K. K.—Pharmacological Action of an Active Constitutent isolated from *Vanda roxburghii* R. Br. (Vol. 34, No. 2).
18. MASON, ELEANOR D., DEVADAS, R., and FRIMODT-MÖLLER, J.—The Effects on Growth in Rats of Butter and Ragi (*Eleusine coracana*), separately and combined, as Supplements to the Poor Rice Diet of South India (Vol. 34, No. 1).
19. MUKERJI, A. K., BHADURI, N. V., and NARAIN, S.—Experiments on the Transmission of Human Schistosomiasis in India (Vol. 34, No. 2).
20. NHAVI, N. G., and PATWARDHAN, V. N.—The Absorption of Fats from the Human Intestine (Vol. 34, No. 1).
21. NHAVI, N. G., and PATWARDHAN, V. N.—The Level of Fat Intake and the Composition of Serum Lipoids (Vol. 34, No. 2).
22. RAO, S. DATTATREYA.—Studies on the Destruction of Vitamin A in Shark-liver Oil. Part VI. Some Factors affecting the Stability (Vol. 34, No. 1).
23. SADANA, J. C., and BASHIR AHMAD.—Observations on the Carotenoid Pigments of local varieties of Yellow Maize (Vol. 34, No. 1).
24. SADANA, J. C., and BASHIR AHMAD.—Observations on the Carotenoid Pigments of the Mango Fruit (Vol. 34, No. 1).
25. SHOURIE, K. L.—Flourine and Dental Caries in India (Vol. 34, No. 1).
26. SHOURIE, K. L.—Eruption Age of Teeth in India (Vol. 34, No. 1).
27. SWAMINATHAN, M.—Vitamin B_1 (Thiamin) Content of Indian Foodstuffs (Vol. 34, No. 2).
28. VEERARAGHAVAN, N.—Studies on the Cultivation of the Aetiological Agent of Rabies *in vitro* and its Nature (Vol. 34, No. 2).
29. VEERARAGHAVAN, N.—Studies on Antirabic Immunization with Culture Vaccine (Vol. 34, No. 2).

B. *Journal of the Malaria Institute of India*

1. COVELL, G.—Malaria Course for Engineers at the Malaria Institute of India, Delhi. Opening address. (Vol. VI, No. 3).
2. COVELL, G.—Malaria and Irrigation in India. (Vol. VI, No. 4).
3. SIDDONS, L. B.—*Anopheles stephensi* as Vector of Malaria in Calcutta (Vol. VI, No. 4).
4. YACOB, M. and SATYA SWAROOP.—Malaria and Rainfall in the Punjab (Vol. VI, No. 3).

C. *List of papers published by workers under the I.R.F.A. in journals other than the Indian Journal of Medical Research and the Journal of the Malaria Institute of India.*

1. BHAGVAT, K.—Interference by Certain Substances in the Estimation of Thiamine by the Thichrome Method (Current Science, Vol. 15, No. 11).

2. BHAGVAT, K. and MISRA, U. C.—A Comparative Study of Acid and Enzymic Extractions of Nicotinic Acid from Foodstuffs (Current Science, Vol. 15, No. 12).

3. DHARMENDRA.—Main Facts about Leprosy (Orissa Junior Red Cross Bulletin, 1946).

4. DHARMENDRA and SEN, N. R.—A Family with 7 Cases of the Neural Type of leprosy (Leprosy in India, Vol. XVIII, No. 2).

5. DHARMENDRA and SANTRA, I.—A Study of the Course of the Disease in Leprosy (Leprosy in India, Vol. XVIII, No. 2).

6. DHARMENDRA and MUKHERJI, N.—Prognostic Value of the Lepromin Test (Leprosy in India, Vol. XVIII, No. 3).

7. DHARMENDRA and SEN, N. R.—Leprosy Bacilli in the Nose of a Neural Case with Bacteriologically Negative Skin Lesions (Leprosy in India, Vol. XVIII, No. 3).

8. GHOSH, L. M. and PANJA, D.—Studies of Ringworm Part III.—Prophylactic measures (Ind. Med. Gaz. Vol. LXXXI, p. 73).

9. GHOSH, L. M. and PANJA, D.—A Case of Granuloma Inguinale in Bengal. (Ind. Med. Gaz. Vol. LXXXI, p. 84).

10. GHOSH, L. M. and PANJA, D.—A Case of Lymphopathia Venereum in Bengal (Ind. Med. Gaz. Vol. LXXXI, p. 137).

11. GOPALAN, C.—The 'Burning-Feet' Syndrome (I.M.G. Vol. LXXXI, No. 1).

12. GOPALAN, C.—Some not well-known Manifestations of Riboflavin Deficiency (I.M.G. Vol. LXXXI, Nos. 6-7).

13. GREVAL, S. D. S., ROY CHOWDHURY, A. B. and BANERJI, B.—Intra group Reaction in an Rh-Expectant Mother & Associated Considerations (Jour. Ind. Med. Assoc. Vol. XV, No. 4).

14. GREVAL, S. D. S., ROY CHOWDHURY, A. B. and BANERJI, B.—A Presumptive Case of Erythroblastosis Foetalis due to Rh Incompatibility. (I.M.G. Vol. LXXXI, Nos. 6-7).

15. PANJA, D. and GHOSH, L. M.—A Case of *Leiomyoma Cutis* (I.M.G. Vol. LXXXI, No. 11).

16. SHOURIE, K. L.—Diet and Dental Caries (J. B. M. A. (Pb. Branch), Vol. XIV, No. 2.

17. SHOURIE, K. L.—Nutrition Problem in the Punjab (J.I.M.A., Vol. XV).

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on 13th and 14th December, 1946.

ENQUIRIES.

		Budget allotments Rs.
	Cholera.	
Continuation enquiries :—		
1.	Cholea treatment unit under the Director, School of Tropical Medicine, Calcutta	32,446
2.	Cholera treatment unit under the Director, King Institute, Guindy, Madras	24,968
3.	Enquiry to prepare monospecific high titre cholera sera under the Director, King Institute, Guindy, Madras	3,168
4.	Enquiry on cholera under the Director, Central Research Institute, Kasauli	24,888
	Malaria.	
Continuation enquiries :—		
5.	Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi	36,282
6.	Mammalian malaria enquiry under the Director, Central Research Institute, Kasauli	8,206
7.	Enquiry on adult *Anopheles stephensi* under the Director, School of Tropical Medicine, Calcutta	9,875
8.	Enquiry on the control of sandflies *Phlebotomus argentipes* by spraying with DDT under Dr. D. N. Roy, at the School of Tropical Medicine, Calcutta	7,875
New enquiries :—		
9.	Enquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta	3,849
10.	Enquiry into the epidemiology of malaria in the Singur Health Unit with particular reference to the problem of transmission under Drs. R. B. Lal, S. C. Seal, and L. M. Bhattacharji of the All-India Institute of Hygiene and Public Health, Calcutta ..	13,714
	Nutrition.	
Continuation enquiries :—		
11.	Nutrition Research Laboratories, Coonoor	1,35,838
12.	Additional nutrition enquiry under Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor ..	36,298
13.	Ascorbic acid enquiry at the University College of Science and Technology, Calcutta	7,200
14.	Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay	3,856

ENQUIRIES.

		Budget allotments Rs.
15.	Nutrition research unit under Dr. H. N. De at Dacca University, Dacca	18,852
16.	Nutrition research unit under Dr. R G. Chitre at the Seth G. S. Medical College, Bombay	16,778
17.	Enquiry on the chemical nature and nutritional availability of food iron at the University College of Science and Technology, Calcutta	4,030
18.	Enquiry on poor rice diets and food preparations in common use in South India under Dr. Eleanor D. Mason at the Women's Christian College, Madras	4,035
19.	Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary proteins to blood and muscle haemoglobin under Prof. M. Damodaran at the University Biochemical Laboratory, Triplicane, Madras	4,140
20.	Enquiry entitled "Preparation of antirachitic substances" under Professor B. V. Bhide at the Maharajah Pratap Sinh Chemical Laboratory, S. P. College, Poona	9,752
21.	Enquiry on the nutritive value of soya-bean milk under Dr. S. S. De at the Indian Institute of Science, Bangalore	7,148

New enquiries :—

22.	Nutrition research unit under Professor V. Subrahmanyan at the Indian Institute of Science Bangalore	18,312
23.	Enquiry on plant carotenoids in relation to their biological importance under Dr. Bashir Ahmad at the Laboratories of the Council of Scientific and Industrial Research, Delhi ..	6,309
24.	Enquiry on the growth promoting factor present in Bengal gram under Dr. Bashir Ahmad at the Laboratories of the Council of Scientific and Industrial Research, Delhi	3,546
25.	Bangalore School Feeding Scheme under Professor V. Subrahmanyan, Professor of Biochemistry, Indian Institute of Science Bangalore	1,000

Leprosy.

Continuation enquiries :—

26.	Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta	16,490

New enquiries :—

27.	Enquiry entitled "Study of histopathology of leprosy" under Dr. R. G. Cochrane, Honorary Director, Leprosy Campaign Madras Presidency, and Principal, Medical College, Vellore ..	15,118

ENQUIRIES.

		Budget allotments Rs.
	Plague.	
	Continuation enquiries :—	
28.	Plague researches under the Director, Haffkine Institute, Bombay	19,085
29.	Plague enquiry at Coonoor in the Nilgiris district under the Director of Public Health, Madras	6,000
	Clinical Research.	
	Continuation enquiries :—	
30.	Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	11,709
31.	Enquiry on Naga sore under Dr. S. R. Pandit at the Pasteur Institute, Shillong	9,380
32.	Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta	13,600
33.	Enquiry into infections of the feet under Rao Bahadur Dr. M. G. Kini, at the Stanley Medical College Hospital, Madras ..	5,807
34.	Dental research unit under Dr. C. D. Marshall Day at the de Montmorency College of Dentistry, Lahore	10,685
35.	Enquiry on the relationship between the measurements of vertebrae and body size of Indians under Dr. R. G. Ginde at the Seth G. S. Medical College, Bombay	3,632
36.	Enquiry into causes of blindness under Captain E. J. Somerset at the Medical College, Calcutta	3,625
37.	Enquiry on typhus under Dr. D. W. Soman at the Haffkine Institute, Bombay	14,208
38.	Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay	9,810
39.	Enquiry on infantile cirrhosis under Dr. P. Kutumbiah at the Stanley Medical College, Madras "	4,294
40.	Enquiry on cirrhosis in animals and its bearing on the aetiology of infantile cirrhosis under Dr. T. Bhaskara Menon at the Andhra Medical College, Vizagapatam	6,435
	New enquiries :—	
41.	Enquiry into clinical and biochemical investigation on certain aspects of infantile hepatic cirrhosis under Dr. V. Subrahmanyan and Professor V. R. Naidu, Professor of Pathology, Medical College, Mysore	9,145

ENQUIRIES.

		Budget allotments Rs.
42.	Enquiry on the effect of vitamins B and C in diabetes under Dr. S. Banerji, Diabetes Research Worker at the School of Tropical Medicine, Calcutta	7,183
43.	Research on lathyrism in the Central Provinces and Berar ..	15,000
44.	Survey of hookworm disease in the Jharia Coalfields Settlement Area under Dr. A. K. Mukerji, Officer-in-Charge, Helminthological Department, School of Tropical Medicine, Calcutta ..	9,421
45.	Enquiry into typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta ...	15,176

Maternity and Child Welfare.

Continuation enquiries:—

46.	Enquiry into anaemia in pregnancy in the Singur Health Unit under Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta	10,550
47.	Enquiry into the bearing of premature and immature births on infant mortality under Dr. B. C. Das Gupta, Executive Health Officer, Bombay Municipality, Bombay	13,031
48.	Enquiry into the pelvic type and its relationship to body build in Indian women under Drs. S. K. Basu, S. Mitra and S. Mukerji at the Carmichael Medical College, Calcutta	2,500

New enquiries:—

49.	Enquiry on anaemias in pregnancy under Dr. R. G. Dhayagude, Professor of Pathology, Seth G. S. Medical College, Bombay ..	6,505
50	Enquiry into the reproductive patterns of urban and rural populations under Dr. C. Chandrasekar, Professor of Statistics and Dr. Muktha Sen at the All-India Institute of Hygiene and Public Health, Calcutta	5,384

Filariasis.

Continuation enquiries:—

51.	Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.	17,117

New enquiries:—

52.	Enquiry to test the usefulness of the intradermal and other tests for the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras	5,626
53.	Enquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona, and Dr. K. Venkatraman, Director of the Technology Department of the University of Bombay	7,304

ENQUIRIES.

		Budget allotments Rs.
	Industrial Health.	
	New enquiries :—	
54.	Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta	30,000
55.	Enquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Professor K. Subrahmanyan, Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, Calcutta	2,700
56.	Enquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under the Chief Medical Officer, Batanagar ..	6,700
57.	Enquiry on fatigue and efficiency in paper and textile industries under Professor Kali Prasad at the Laboratories of Experimental Psychology, Lucknow University	6,860
	Pharmacology.	
	Continuation enquiries :—	
58.	Indigenous drugs enquiry under Bt.-Col. Sir Ram Nath Chopra, C.I.E., I.M.S., (R) at the Drug Research Laboratory, Jammu, Kashmir	6,722
	Other Researches.	
	Continuation enquiries :—	
59.	Haematological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	12,447
60.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	11,506
61.	Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science and Technology, Calcutta ..	3,764
62.	Enquiry on kala-azar under Dr. P. C. Sen Gupta at the School of Tropical Medicine, Calcutta	8,820
63.	Helminthological enquiry under Dr A. K. Mukerji at the School of Tropical Medicine, Calcutta	7,167
64.	Enquiry into methods for the manufacture of penicillin and other problems connected with it under the Director, Haffkine Institute, Bombay	17,642
	New enquiries :—	
65.	Enquiry to investigate allergic diseases and significance of endogenous allergens as regards ætiology, diagnosis and treatment	

ENQUIRIES.

		Budgts allotments Rs.
	of allergic diseases under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta	4,849
66.	Enquiry on the enteric group of fevers under Dr. R. N. Chaudhuri, Officiating Professor of Tropical Medicine, School of Tropical Medicine, Calcutta	4,900

GRANTS.

(i)	Office of the Secretary, Indian Research Fund Association ..		45,661
(ii)	Staff employed in the office of the Accountant-General, Central Revenues for I.R.F.A. work		7,043
(iii)	I.R.F.A. publications		4,500
(iv)	Travelling allowance of the members of the Governing Body, Scientific Advisory Board and Advisory Committees of the I.R.F.A.		12,000
(v)	Contribution towards the cost of two professorships at the School of Tropical Medicine, Calcutta		61,356
(vi)	Contribution to the Imperial Institute of Entomology, London		1,350
(vii)	Contribution towards the Bureau of Hygiene and Tropical Diseases, London		2,025
(viii)	Research fellowships		52,000
(ix)	Indian Journal of Medical Research and Indian Medical Research Memoirs		
	(a) Printing and publication charges of the Journal	14,000	
	(b) Printing and publication charges of the Memoirs	2,000	
	(c) Editor's office	8,903	
			24,903
(x)	I.R.F.A. library at the Central Research Institute, Kasauli ..		2,461
(xi)	I.R.F.A. Microfilm Service Unit at the Central Research Institute, Kasauli		13,520
(xii)	Pay (including special pay, overseas pay and allowances) and contributions towards passage, leave salary and pension or contributory provident fund of officers of the Medical Research Department under the Association		75,000
(xiii)	Travelling allowances and certain contingent expenses for Medical Research Department officers who are employed under the I.R.F.A. and are attached to Central or Provincial Laboratories		10,000
(xiv)	Pay and travelling allowance of Junior Research Worker ..		10,532
(xv)	Clerical assistance at the School of Tropical Medicine, Calcutta		5,479
(xvi)	Clerical assistance to the Director, All-India Institute of Hygiene and Public Health, Calcutta		1,683

ENQUIRIES.

		Budget allotments Rs.
(xvii)	Travelling allowance for I.R.F.A. workers on transfer ..	3,000
(xviii)	I.R.F.A. contribution and interest towards the Contributory Provident Fund	17,000
(xix)	Miscellaneous unforseen expenditure	50,000
(xx)	Imprest advance for enquiries	10,790
(xxi)	Printing and publication charges of the Journal of the Malaria Institute of India	12,100

JUN. 1948

REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR 1947

Issued under the authority of the Governing Body,
Indian Research Fund Association,
New Delhi,
India.

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I. The Scientific Advisory Board consisted of the following :—

Chairman :

Lieut.-General R. Hay, C.I.E., K.H.P., I.M.S., Director-General, Indian Medical Service (1st January to 14th August, 1947).

Dr. Jivraj N. Mehta, M.D., M.R.C.P., Director-General of Health Services, Government of India (15th August to 31st December, 1947).

Secretary :

Colonel E. Cotter, C.I.E., V.H.S., I.M.S., Public Health Commissioner with the Government of India (1st January to 25th April, 1947).

Lieut.-Colonel M. Jafar, O.B.E., I.M.S., Public Health Commissioner with the Government of India (23rd May to 5th August, 1947).

Lieut.-Colonel T. C. Puri, M.B.B.S., D.P.H., D.T.M. & H., Deputy Director-General of Health Services, Government of India (15th August to 31st December, 1947).

Members :

Lieut.-Colonel H. W. Mulligan, I.M.S., Director, Central Research Institute, Kasauli (1st January to 1st April, 1947).

Lieut.-Colonel M. L. Ahuja, M.B., Ch.B., M.D., D.P.H., Director, Central Research Institute, Kasauli (8th May to 31st December, 1947).

Lieut.-Colonel C. L. Pasricha, I.M.S., Director, School of Tropical Medicine, Calcutta (1st January to 14th August, 1947).

Dr. D. N. Roy, M.D., D.T.M., Director, School of Tropical Medicine, Calcutta (15th August to 31st December, 1947).

Major-General Sir Sahib Singh Sokhey, M.A. (Edin.), B.Sc. (Punj.), M.B., Ch.B. (Edin.), M.D. (Edin.), D.T.M. & H. (Eng.), F.A.S., F.N.I., Director, Haffkine Institute, Bombay.

Major-General Sir Gordon Covell, C.I.E., K.H.P., I.M.S., Director, Malaria Institute of India, Delhi (1st January to 28th February, 1947).

Lieut.-Colonel M. K. Afridi, I.M.S., Director, Malaria Institute of India, Delhi (1st March to 9th August, 1947).

Lieut.-Colonel Jaswant Singh, M.B., Ch.B. (Edin.), D.T.M. & H. (Lond.), D.P.H. (Eng.), Director, Malaria Institute of India, Delhi (18th August to 31st December, 1947).

Dr. C. G. Pandit, O.B.E., M.B.B.S., Ph.D., D.P.H., D.T.M., Director, King Institute, Guindy, Madras.

Lieut.-Colonel C. K. Lakshmanan, L.M. & S., M.R.C.S., D.T.M. & H., D.P.H. (Eng.), Director, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. Bashir Ahmad, M.Sc., Ph.D. (Lond.), F.N.I., Director, University Institute of Chemistry, Punjab University, Lahore.

Dr. A. N. Goyle, M.D., Ph.D., Principal, Glancy Medical College, Amritsar.

Dr. B. C. Guha, D.Sc., F.N.I., Professor of Biochemistry, University College of Science & Technology, 92 Upper Circular Road, Calcutta.

Professor S. W. Hardikar, M.D., M.R.C.P., 'Abhas', Hardikarbagh, Himayatnagar, Hyderabad (Deccan).

Dr. V. R. Khanolkar, B.Sc. (Lond.), M.D. (Lond.), Director of Laboratories, Tata Memorial Hospital, Bombay.

Dr. Jivraj N. Mehta, M.D., M.R.C.P., Khambatta Hill, 16 Altamont Road, Bombay (1st January to 14th August, 1947).

Co-opted member :

Dr. (Miss) S. Pandit, W.M.S., Director of Maternity & Child Welfare Bureau, Indian Red Cross Society, New Delhi.

II. Composition of Advisory Committees for the year 19

1. *Cholera.*

Major-General Sir Sahib Singh Sokhey. (*Chairman*).
Lieut.-Colonel M. L. Ahuja.
Dr. B. B. Dikshit.
Dr. J. C. Gupta.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Lieut.-Colonel C. L. Pasricha.
Rao Bahadur Dr. K. C. K. E. Raja.
Dr. D. L. Shrivastava.
Dr. K. V. Venkatraman. (*Secretary*).

2. *Malaria.*

Lieut.-Colonel Jaswant Singh. (*Chairman*).
Dr. B. Ananthaswamy Rao.
Rai Bahadur Dr. A. C. Banerjea.
Bt.-Colonel Sir Ram Nath Chopra.
Dr. M. O. T. Iyengar.
Dr. K. V. Krishnan.
Dr. P. Kumaran Nair.
Dr. D. N. Roy.
Dr. D. K. Viswanathan.
Dr. K. S. Viswanathan.
Dr. I. M. Puri.
Dr. S. P. Ramakrishnan. } (*Joint Secretaries*).

3. *Nutrition.*

Dr. G. Sankaran. (*Chairman*).
Dr. B. C. Guha.
Mr. B. N. Adarkar.
Professor S. P. Agharkar.
Dr. Bashir Ahmad.
Dr. S. Banerji.
Dr. K. P. Basu.
Dr. C. O. Karunakaran.
Dr. R. B. Lal.
Dr. T. Bhaskara Menon.
Dr. V. N. Patwardhan. (*Secretary*).

4. *Plague.*

Major-General Sir Sahib Singh Sokhey. (*Chairman*).
Dr. P. V. George.
Dr. A. N. Goyle.
Lieut.-Colonel S. D. S. Greval. (*Co-opted member*).
Dr. V. R. Khanolkar.
Dr. R. B. Lal.
Dr. C. G. Pandit.
Dr. M. Sharif.
Dr. K. V. Venkatraman.
Dr. P. M. Wagle. (*Secretary*).

5. *Clinical.*

Dr. V. R. Khanolkar. (*Chairman*)
Dr. R. N. Choudhuri.
Dr. R. N. Cooper.
Dr. C. R. Das Gupta.
Dr. A. N. Goyle.
Dr. S. W. Hardikar.
R.B. Dr. M. G. Kini.
Dr. P. Kutumbiah.
Lieut.-Colonel S. Narain.
Dr. J. C. Ray.
Dr. B. B. Yodh.
Dr. J. C. Patel. (*Secretary*).

6. *Rabies.*

Lieut.-Colonel M. L. Ahuja. (*Chairman*).
Dr. K. Banerjee.
Dr. C. B. D'Silva.
Lieut.-Colonel S. D. S. Greval.
Dr. S. R. Pandit.
Dr. R. Sanjiva Rao.
Dr. N. Veeraraghavan.
Dr. K. V. Venkatraman.
Dr. A. G. Brooks. (*Secretary*).

7. *Leprosy.*

Dr. C. G. Pandit. (*Chairman*).
Lieut.-Colonel A. N. Chopra.
Dr. R. G. Cochrane.
Dr. P. V. Gharpure.
Dr. V. R. Khanolkar.
Dr. Dharmendra. (*Secretary*).

8. *Filariasis.*

Dr. C. G. Pandit. (*Chairman*)
Dr. R. G. Dhayagude.
Dr. B. B. Dikshit.
Dr. A. K. Mukerji.
Lieut.-Colonel C. L. Pasricha.
Lieut.-Colonel Jaswant Singh.
Dr. M. O. T. Iyengar. " (*Secretary*).

9. *Industrial Health.*

Lieut.-Colonel C. K. Lakshmanan. (*Chairman*).
Dr. B. C. Das Gupta.
Major E. L. Jones.
Mr. S. C. Joshi.
R.B. Dr. M. G. Kini.
Mr. N. S. Mankiker.
Lieut.-Colonel T. C. Puri.
Major J. Sen.
Professor K. Subrahmanyan.
Dr. M. N. Rao. (*Secretary*).

10. *Maternity and Child Welfare.*

Dr. J. Jhirad. (*Chairman*).
Dr. A. Benjamin.
Miss M. Craig.
Dr. C. R. Das Gupta.
Dr. J. M. Orkney.
Dr. N. A. Purandare.
Rao Bahadur Dr. K. C. K. E. Raja.
Dr. Muktha Sen.
Dr. S. Pandit. (*Secretary*).

III. Meetings held by the Scientific Advisory Board in 1947.

The Board held its annual meeting on the 12th and 13th December, 1947 and confirmed the composition of the various Advisory Committees which had been agreed to by the circulation of papers, and decided on the co-option of experts for its annual meeting. It also considered in detail the programme of research for the year 1948-49 and recommended the programme given on pages 204 to 209 of this report.

TECHNICAL REPORT OF THE SCIENTIFIC ADVISORY BOARD OF THE INDIAN RESEARCH FUND ASSOCIATION FOR THE YEAR 1947.

The researches which were carried out during the year under report were recommended by the Board at its meeting held in Bombay on the 13th and 14th December, 1946 and were approved by the Governing Body of the Indian Research Fund Association at its meeting held in New Delhi on the 19th March, 1947.

IV. Researches carried out during the year.

CHOLERA

1. Cholera treatment unit under the Director, School of Tropical Medicine, Calcutta.

A. *Field trials.*—During this year two field investigations, one in Faridpur in East Bengal and the other in Dumraon in Bihar, were undertaken to test the action of sulphaguanidine in the treatment of cholera.

(1) Faridpur: The outbreak occurred in April. Altogether 16 cases were treated with sulphaguanidine out of which 2 (12·5%) died and out of a parallel series of 14 control cases 4 (28·5%) died. The control cases were treated in different ways. Some were treated by homeotherapy, some were given saline injection but none of these cases was given sulphaguanidine.

(2) Dumraon: The outbreak occurred in September. A total of 26 cases of cholera were treated with this drug out of which 2 (7·6%) died, whereas out of 19 control cases 9 (52·6%) died.

The striking feature observed in the treatment of cholera with sulphaguanidine is the fact that this drug acts best during the early stages of the disease, i.e. during diarrhœa and pre-collapsed stage. In Dumraon 11 cases were treated with sulphaguanidine within a short time of the onset of the disease and in none of them was saline injection necessary. All were cured by this drug alone. Out of these 11 cases 8 were examined bacteriologically and vibrios were isolated from 6 of them on stool-culture. All the above cases were treated in ordinary rural surroundings and the patients lived in *bustis*.

B. *Therapeutic trials in Calcutta.*—Sulphaguanidine, sulphadiazine and phthalylsulphathiazole were tried during the year under review. The doses given and the selection of cases were on the same lines as were reported previously. The results of trials during this year are tabled below:—

	Cases.	Deaths.
TABLE I.		
Sulphaguanidine ..	404	24 or 5·9%
Control ..	410	41 or 10%
TABLE II.		
Sulphadiazine ..	518	48 or 9·3%
Control ..	525	58 or 11%
TABLE III.		
Phthalylsulphathiazole ..	320	22 or 6·9%
Control	331	31 or 9·4%

From the above it will appear that in all the 3 groups, i.e. sulphaguanidine, sulphadiazine and phthalylsulphathiazole, the mortality rate was lower than in control series but excepting the results of sulphaguanidine, none of the other two was statistically significant.

C. *Laboratory*—(1) *Report of the examination of stool :*

No. of cases examined.	Vibrio positive.	(Per cent positive).	Ogawa.	Inaba.
838	531	63·4	115 (21·1%)	416 (78·4%)
808	256	31·7	2 (0·8%)	254 (99·2%)

The above cases have been grouped into two different series for the reason that on the first series the agar out of which media were prepared was of good quality, whereas in the second series, the agar was of poor quality. This accounts for a lower percentage of vibrio positives.

(2) *Estimation of chloride and protein in the blood of cholera cases :* Arrangements are almost complete for the estimation of cholride and protein contents of the blood of cholera patients. The estimation of the former is necessary for the purpose of determining the appropriate strength of sodium chloride of the saline solution used for intravenous injections. The object of the estimation of protein is to find out if any protein is actually depleted with evacuations. Some workers have claimed a definite improvement in cholera when patients are treated with plasma transfusion.

2. Cholera treatment unit under the Director, King Institute, Guindy, Madras.

Two separate investigations are now in progress to assess the value of sulphaguanidine in the treatment of cholera—one at the Infectious Diseases Annexe of the Government Erskine Hospital, Madura, and the other at the Government Headquarters Hospital, Trichinopoly. Complete understanding has been established between the staff of the hospitals and the members of the investigating team, thanks to the keen interest shown by the Superintendents of these hospitals. A serious handicap in the treatment of cholera in these and other district hospitals has been the lack of adequate facilities for the preparation of large quantities of sterile pyrogen-free transfusion fluids. This has been overcome for the present by the preparation of these fluids at this Institute and their despatch at regular intervals to the field units in standard blood transfusion bottles. In this manner over 550 litres of sterile normal saline and 5% glucose saline have been supplied and their use has resulted in the total elimination of febrile reactions following transfusions which used to be such a common feature before.

The procedure adopted is briefly as follows :—

On admission, cases are assigned alternately for treatment by the routine methods and treatment with sulphaguanidine. A brief record is made of the history and the condition on admission and includes the blood specific gravity as determined by the copper-sulphate method, and blood pressure. A specimen is obtained for bacteriological confirmation of the diagnosis, generally by the insertion of a sterile swab in the rectum and inoculation of KCl-boric-buffer solution and a Rileys slant, which are then

mailed to the Institute. Administration of saline is controlled by the blood-specific gravity as well as by the pulse and general reaction. Routine treatment consists of the administration of an essential oil mixture, pot. permanganate pills and bismuth-kaolin mixture. Cases assigned for sulphaguanidine treatment receive 5 grammes of sulphaguanidine immediately and 0·5 g. every hour thereafter till discontinued. Frequent administration of the drug in divided doses has resulted in less vomiting than when the drug was given in larger doses every 4 hours. Other supportive treatment and repetition of transfusions are determined by the progress of the patient and are common to both groups. Adequate records are maintained of the progress.

The specimens of stools received in the laboratory, usually after a delay of about 2 to 3 days, were in the beginning plated both on Aronson plates and modified Rileys solid medium. A review of the results obtained with the first 265 specimens showed the superiority of the modified Riley's medium and plating on Aronson was dropped from 30th September, 1947.

Generally the correlation between the results of cultural examination and the clinical diagnosis has been satisfactory.

A preliminary analysis of the end results of 188 consecutive culture-positive cases treated in the Madura hospital shows that in 93 cases under routine treatment there were 16 deaths, while in 95 cases treated with sulphaguanidine there were 17 deaths. A more detailed analysis similar to that made previously will be done when the investigation is completed.

Earlier in the year, the staff of the Unit were engaged in the study of the nutritional requirements of *V. cholerœ*. This work has been suspended and will be resumed at a later date.

3. Enquiry to prepare monospecific high-titre cholera sera under the Director, King Institute, Guindy, Madras.

The preparation of monospecific agglutinating sera for Inaba and Ogawa types of *V. cholerœ* was continued and requests for these from other investigators met. Approximately 150 ml. of monospecific Inaba serum and 250 ml. of Ogawa serum of satisfactory titre are still available for distribution. It is not proposed to continue this enquiry.

4. Enquiry on cholera under the Director, Central Research Institute, Kasauli.

During the period under review, in collaboration with Mr. P. Bruce White in the earlier months of the year, work was undertaken on the serological variations of *V. cholerœ* and El Tor vibrios under the stimulus of type specific serum with and without the addition of fresh compliment. The following observations are recorded :—

(1) When strains of the Ogawa type of *V. cholerœ* and El Tor vibrios are grown in broth containing type homologous monospecific serum, they quite readily yield cultures of the Inaba type.

(2) It has not yet been possible to induce a change in the classic Inaba strains to Ogawa type by growing them in the presence of Inaba type specific serum or any other means tried so far.

(3) Certain strains belonging to the "Intermediate" category, predominantly Inaba type in serology but containing also an imperfect

Ogawa factor have been shown to yield races indistinguishable from cultures of the Ogawa type under the stimulus of Inaba type monospecific serum.

These findings have been published in the July 1947 issue of *Indian Journal of Medical Research.*

Although the exact mechanism of the change of Ogawa into Inaba is not clear it is possibly due to failure of the Ogawa organism to synthesize certain chemical groupings when subjected to the influence of its homologous antibodies. The real explanation will perhaps be found by immuno-chemical studies of the antigens of the various types of vibrios. Inaba being simpler in structure will have to be subjected to a process of synthesis, probably enzymatic, in order that the change in the reverse direction may be brought about.

Besides the above a considerable amount of work has been done on the isolation of fractions including Cα and Cβ of Bruce White from various strains of vibrios. These are awaiting investigation.

List of papers published.

ADISESHAN, R., PANDIT, C. G., and VENKATRAMAN, K. V.—Statistical Evaluation of Anti-cholera inoculation as a Personal Prophylactic against Cholera and its Efficacy in the Prevention and Control of Cholera Epidemics. (*Ind. Jour. Med. Res.*, Vol. **35**, No. 3).

CHANDRA SEKAR, C.—Statistical Assessment of the Efficacy of Anti-cholera Inoculation from the Data of 63 *Cheris* in South Arcot District. (*Ind. Jour. Med. Res.*, Vol. **35**, No. 3).

PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS, A. K.—Sulphaguanidine in the treatment of cholera (*Ind. Med. Gaz.*).

PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS, A. K.—Sulphadiazine in the treatment of cholera (*Ind. Med. Gaz.*).

PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS, A. K.—Sulphasuxidine in the treatment of cholera. (*Ind. Med. Gaz.*).

PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS, A. K.—Phthalylsulphathiazole in the treatment of cholera (*Ind. Med. Gaz.*).

SHRIVASTAVA, D. L., and BRUCE WHITE, P.—Note on the relationship of the so-called Ogawa and Inaba types of *V. cholerœ* (*Ind. Jour. Med. Res.*, Vol. **35**, No. 3).

REPORT OF THE CHOLERA ADVISORY COMMITTEE.

The Committee met at 2-30 p.m. on Tuesday the 9th December, 1947, in the Imperial Secretariat, New Delhi.

MEMBERS PRESENT.

Major General Sir Sahib Singh Sokhey. (*Chairman*).
Lieut.-Colonel M. L. Ahuja.
Dr. B. B. Dikshit.
Dr. J. C. Gupta.
Dr. C. G. Pandit.
Dr. S. R. Pandit.
Rao Bahadur Dr. K. C. K. E. Raja.
Dr. D. L. Shrivastava.
Dr. K. V. Venkatraman. (*Secretary*).

Lieut.- Colonel C. L. Pasricha was unable to attend.

The following also attended :—

Lieut.-Colonel Barkat Narain.
Major-General A. C. Chatterji.
Lieut.-Colonel A. N. Chopra.
Lieut.-Colonel A. N. Duggal.
Major N. Jungalwalla.
Dr. M. N. Lahiri.
Lieut.-Colonel C. Mani.
Dr. A. R. Mehta.
Dr. D. R. Mehta.
Lieut.-Colonel T. C. Puri.
Dr. N. Veeraraghavan.

1. The Committee considered the work carried out by the following enquiries. :—

(i) *Cholera treatment unit under the Director, School of Tropical Medicine, Calcutta.*

Dr. J. C. Gupta in presenting the report of the work done by the unit stated that two field investigations comprising 30 cases and 45 cases respectively were carried out during the year. Sulphaguanidine was administered to 16 cases in the first group and 26 cases in the second, with two deaths in each; in 14 control cases of the first group there were 4 deaths and 9 in the 19 of the second group. In therapeutic trials carried out in the hospitals in Calcutta, sulphaguanidine, sulphadiazine and phthalylsulphathiazole were tried. In a total of about 2,500 cases, in all the three groups the results showed that the mortality rates were slightly lower than in the control series and excepting in the case of sulphaguanidine the results were not, however, statistically significant. He stated that the arrangements have been completed for the estimation of chloride and protein contents of the blood of cholera patients.

(ii) *Cholera treatment unit under the Director, King Institute, Guindy, Madras.*

Dr. K. V. Venkatraman, presenting the report of the work done by the unit, gave an account of the investigations recently carried out at the H.Q.

Hospitals at Madura and Trichinopoly. The Madura group comprised a series of 404 cases admitted to the cholera wards of the hospital between 1st October and 17th November, alternate cases being treated with sulphaguanidine and essential oils respectively, the latter being the routine of the hospital. All cases received saline transfusions for the control of de-hydration and collapse and other supportive treatment as indicated. Specimens were obtained from each case for bacteriological confirmation of the diagnosis. Careful records have been maintained of the condition of the patients at the commencement of treatment and the progress of the cases and these await analysis. Taking only the end result, death or recovery, as a criterion of the efficacy of treatment, in 278 consecutive culturally positive cases, there were 20 deaths in 139 cases in the sulphaguanidine group and an equal number (20) in the remaining 139 cases of the control group. The figures for the whole series were 27 deaths in 198 cases treated with sulphaguanidine and an equal number in 206 cases of the control. Dr. Venkatraman wished to draw attention to the comparatively low case mortality rate of 13·3%—much lower than it has been the experience in previous years, and the general case mortality rates recorded in the districts during the present outbreak—and attributed it mainly to the provision of pyrogen-free transfusion fluids and more rational transfusion methods.

The Trichinopoly group consisted of a smaller series of 60 cases admitted to the cholera wards of the hospital between 7th October and 30th November. A diagnosis of cholera was excluded in 11 cases and of the remaining 49 cases *V. cholerœ* was isolated from 42. There were 7 deaths among 23 cases treated with sulphaguanidine and 9 deaths among the 26 cases in the control group.

These results showed that in hospitalized cases of cholera receiving adequate saline transfusion and other supportive treatment, administration of sulphaguanidine does not improve the results in the treatment of cholera.

Dr. Venkatraman further reported that a trial of the formalinsulphanilamide compound prepared by Dr. Dikshit has been commenced in the Madura hospitals, where a fair number of cases of cholera continue to be received and hoped that sufficient material for a statistical evaluation of the drug would be available in a short time. A report on this will be communicated when the investigation is completed.

There was a lively discussion on the subject. Colonel M. L. Ahuja expressed the desirability of carrying out these comparative trials under conditions obtaining in the rural areas, where hospitalization and transfusions are not practicable. Dr. R. B. Lal pointed out that it is important to compare cases in clinically similar stages when assessing the merits of two treatments; and since the two treatments may act in different ways, they may be more or less effective in different stages of the disease. The Directors of Public Health present at the meeting expressed the view that it is important to conduct the investigations under rural conditions and a remedy, which under these conditions would reduce cholera mortality, would be of great practical importance. General Sokhey suggested that temporary hospitals may be organized in cholera-affected villages where such trials could be conducted under controlled conditions. Dr. C. G. Pandit pointed out that, under those conditions, it will not be possible to withhold saline transfusions and the results are not likely to be any more conclusive than the trials in established hospitals with better facilities.

Dr. Raja stated that for the purpose of establishing the efficacy of a remedy it was essential that critical trials should be conducted under strictly controlled conditions which excluded extraneous factors and, when the efficacy has been established, it may be released for use in the rural areas. He, however, agreed that, as saline transfusions are impracticable under rural conditions where most of the cases of cholera occurred, it may be desirable to ascertain if the administration of the drug would reduce the high mortality to a level which obtains in hospitalized cases receiving saline transfusion without the benefit of a specific remedy.

Dr. Venkatraman referred to a limited trial carried out in a rural area in the Madura district in 1943. A medical officer was stationed in the village to whom all cases of cholera were notified as they occurred. He visited the cases in their homes, made a record of the condition of the patient, obtained a specimen of stool for cultural examination and administered sulphaguanidine or a placebo alternately, and left subsequent doses of the drug (or placebo) for subsequent administration at prescribed intervals. An attendant of the enquiry visited the homes fairly frequently to ascertain if subsequent doses had in fact been given and the medical officer made at least two visits daily to record progress. No saline was administered to any of the cases. Dr. Venkatraman did not have the figures at hand, but the experience covered about a hundred cases, the cultural examination of the stools at the Institute gave a remarkably high correlation with the clinical diagnosis in the field and the results were not strikingly different in the sulphaguanidine and control groups. He considered these precautions to be the minimum requisite if any valid conclusions were to be drawn from investigations under rural conditions. Proper selection of villages, based on continuous contact with health intelligence, giving a high probability of the occurrence of fairly large numbers of cases in compact village groups, was essential to secure data adequate for statistical evaluations. Such opportunities will not be rare during epidemic prevalence of cholera.

The Committee were of the opinion that the amount of work already done in hospitals, where these drugs had been administered in conjunction with saline transfusions and other supportive treatment, indicated that they do not appear to have any special value under these conditions and that further trials under such conditions may be discontinued. They desire that further trials should be restricted to rural areas under conditions described by Dr. Venkatraman. As favourable conditions for such an enquiry are likely to be present in the Madras Province, they recommend that the treatment unit at the King Institute be continued and the grant of Rs. 30,967 asked for be sanctioned.

The Committee do not recommend the continuation of the Cholera Treatment Unit at the School of Tropical Medicine, Calcutta, in its present form. The enquiry may be restricted to a biochemical investigation of the cases of cholera in Calcutta hospitals and that the workers should also undertake the preparation and supply of monospecific sera which is being discontinued at Madras. They recommend that the Director of the School be requested to submit a revised proposal for a grant not exceeding Rs. 10,000 for this purpose. It is expected this would absorb the senior research worker who has been continuously with the Association for a number of years. The Chairman appealed to Major-General Chatterji, Director of Public Health, West Bengal, who was present, to make every possible effort to absorb the other workers of the enquiry in the public health work of the province. Major-General Chatterji said that he

would be very happy to do everything possible and recommended that the Association should get in touch with him in this regard.

(iii) *Enquiry to prepare monospecific high-titre cholera sera under the Director, King Institute, Guindy, Madras.*

Dr. C. G. Pandit stated that the enquiry continued to prepare monospecific sera and had met all demands made during the year. Approximately 150 ml. of monospecific Inaba serum and 250 ml. of Ogawa serum were still available for distribution. He did not propose to continue the enquiry.

(iv) *The cholera enquiry under the Director, Central Research Institute, Kasauli.*

Colonel M. L. Ahuja presented the report of the work done on this enquiry and stated that they had succeeded in inducing mutation of Ogawa type of *V. cholerœ* and El Tor vibrios by growing them in the presence of Ogawa type-specific serum, but it has not been found possible to induce similar change in the Inaba type. The exact mechanism of the change was not quite clear and it was probably due to the failure of the Ogawa organism to synthesize certain chemical groupings when subjected to the influence of its homologous antibodies. A considerable amount of work has also been done on the isolation of several fractions from various strains of vibrios.

Colonel Ahuja agreed to reduce the budget by Rs. 2,500 under the head chemicals and apparatus. The Committee recommended the enquiry be continued and that a grant of Rs. 20,266 be sanctioned for this purpose.

2. Consideration of section 2(iv) of the minutes of the 7th meeting of the Medical Research Liaison Committee for India held in March 1947.

Noted.

3. Consideration of section 3 of the minutes of the 8th meeting of the Medical Research Liaison Committee for India held in June 1947.

Noted.

4. Consideration of a note on the "Re-appearance of the Inaba type of *Vibrio cholerœ* in the epidemic of Cholera in the Madras Province" by Dr. K. V. Venkatraman (Appendix I).

Dr. K. V. Venkatraman in presenting his very interesting note remarked that, from the records he has maintained at the King Institute, Guindy, Madras, relating to the types of *V. cholerœ* isolated from specimens submitted to the Institute and the places of origin of these strains, it was found that during the years 1936 to 1938 there was a clear demarcation of the areas of the prevalence of the two types. Following 1939, the year of the lowest incidence of cholera, Ogawa vibrios became predominant in the cholera epidemics during the period 1939 to 1946. The severe epidemic in 1942-44 was entirely of the Ogawa type. 1946 was again a year of low incidence and it is of interest to note that subsequent to this period the Inaba type of vibrio has again made its appearance and largely became predominant during the present severe epidemics of cholera. He made a suggestion that the change in the prevalent type may be significant in the cyclical periodicity of epidemic cholera. It would facilitate a more correct appraisal of the significance if similar data were available for other parts of India. The Committee considered these suggestions of great interest and desired that the Directors of Public Health and Directors of Laboratories should be requested to gather similar information in their respective provinces.

5. Note on a comparative test of the antigenic potency of two specimens of Cholera vaccine prepared by different methods, by Dr. K. V. Venkatraman (Appendix II).

In the absence of facilities for repeating Major-General Sokhey's method in the comparison of a specimen of casein-hydrolysate vaccine and agar vaccine, a direct comparison of the vaccine was made by determining the L.D. 50 of a virulent culture in two groups of mice given optimal doses of the vaccine. There was no significant difference in the protective value of these two vaccines. The mice receiving either of these vaccines withstood approximately 100 times more of the challenge culture than unprotected mice. It was pointed out that the two vaccines used in this test were about two years old and it would be desirable to repeat these with fresh vaccines and by the two methods simultaneously.

6. Major-General Sokhey brought to the notice of the Committee that in the recent widespread cholera epidemic in Egypt large quantities of cholera vaccine prepared by different institutions in many ways were used. This has raised the question of devising an appropriate method to determine the suitability of a cholera vaccine for use. This question has been referred to the Expert Committee on Biological Standardization of the World Health Organization of which Major-General Sir Sahib Singh Sokhey is a member. Among the possible tests are the suitability of strains used, the opacity of vaccine and the mouse-protection tests. This mouse-protection test was accepted by this Committee last year but we have still no information as to the relation of this test to the protection in human beings. As India is in a position to make this information available, Major-General Sokhey suggested that we should undertake a controlled field trial. Such a field trial will be of utmost value and importance to both this country and the World Health Organization.

The Committee, therefore, recommends that, in view of the facilities available in Madras Province, the Director of Public Health, Madras, and the Director, King Institute, Guindy, Madras, be requested to undertake this investigation on planned basis in a suitable area in the province, and that a grant of Rs. 20,000 be made by the I.R.F.A. for this purpose, and the Director, Haffkine Institute, Bombay, may be requested to provide necessary amount of casein-hydrolysate vaccine free of charge.

7. Major-General Sokhey drew attention to the great importance of the discussions on cholera that were likely to take place at the forthcoming International Congress of the Tropical Medicines to be held in Washington in May next year because of the recent extension of cholera epidemic in the Middle East and Egypt. This outbreak of cholera has made cholera a burning international question. As the discussions would be of the greatest importance and value to science and this country, it was his opinion that India should be strongly represented at these discussions.

The Committee agreed with the views and recommends that the Government of India may be addressed on the subject to send a delegation to represent India to enable the research workers to contribute from their experience on cholera research that has been carried out under the auspices of the Indian Research Fund Association during recent years.

APPENDIX I.

A note on the reappearance of the Inaba type of *V. cholerœ* in the Madras Province by K. V. Venkatraman.

In a previous note submitted to the Committee in 1945 on the distribution of the types of *V. cholerœ* in the Madras Province it was shown that following a year of low incidence in 1939 a change occurred in the prevalent type of the vibrio. During the period 1939-46, which included the widespread epidemics of 1942-43, Ogawa vibrios were almost exclusively prevalent throughout the province. 1946 has been a year of very low incidence comparable to 1939, and it is of interest to record that a change has again occurred in the prevalent type. Almost all the vibrios isolated during the present outbreak beginning July 1947, have been of the Inaba type. The accompanying table shows the number of strains of *V. cholerœ* of each type isolated from specimens sent to the Institute during each year from 1936 and the recorded incidence of cholera in the different regions during the corresponding period.

This note is now presented as a record of observation; and the suggestion is made that the change in the prevalent type may be significant in the cyclical periodicity of epidemic cholera.

TABLE.

Region	1936		1937		1938		1939		1940		1941		1942		1943		1944		1945		1946		1947*	
	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa	Inaba	Ogawa
N. Circars ..	58	4	106	2	102	1	0	0	22	241	1	115	0	154	0	75	0	176	1	260	2	15	67	3
	..		..		(4,451)		(269)		(3,288)		(4,499)		(1,620)		(9,447)		(11,657)		(10,144)		(1,166)		..	
Ceded districts ..	18	14	87	10	60	1	0	16	0	8	0	17	0	13	3	13	0	8	0	0	0	62	6	0
	..		..		(3,861)		(107)		(158)		(270)		(13,694)		(7,592)		(1,817)		(6,054)		(168)		..	
East Central ..	59	312	952	127	91	1	0	3	0	2	0	3	7	255	17	282	0	100	0	116	0	4	25	2
	..		..		(1,569)		(65)		(68)		(122)		(980)		(27,530)		(6,066)		(1,151)		(49)		..	
S. districts ..	11	228	8	177	23	11	0	29	0	1	2	21	2	213	2	131	2	48	0	6	0	2	576	6
	..		..		(3,558)		(1,184)		(111)		(139)		(8,285)		(26,108)		(11,032)		(316)		(327)		..	
West & West Central ..	5	57	44	84	0	0	0	0	0	1	0	0	0	9	18	9	0	37	0	6	0	0	27	5
	..		..		(337)		(20)		(28)		(33)		(727)		(43,877)		(4,252)		(373)		(130)		..	
Madras Province	151	615	1197	400	276	14	0	48	22	253	3	166	9	644	40	510	2	369	1	388	2	83	701	16
	..		..		(13,776)		(1,665)		(3,653)		(5,063)		(25,306)		(114,554)		(34,724)		(18,038)		(1,840)		..	

Fig. in brackets :—Total No. of attacks. *1947–11 months.

APPENDIX II.

A comparative test of the antigenic potency of two specimens of cholera vaccine prepared by different methods.

Major-General Sokhey has described a test of potency of anti-cholera vaccine in which groups of mice given varying doses of the vaccine in two spaced injections are challenged subsequently with approximately 10 M.L.D. of virulent test culture and the protective dose of the vaccine estimated by the 50% mortality end-point. The challenge dose has since been increased to 100 M.L.D. A comparison of different lots of cholera vaccine is made possible by the determination of the protective dose of each. This method of comparison of vaccines by the determination of what has been called their antigenic-extinction titre is particularly applicable to those cases where the indicator system lends itself to the demonstration of repeatable end-point. In a slightly modified form this has been used in the case of influenza virus vaccines by Blaskovie and Salk.

A more direct method of comparison of two lots of cholera vaccine is possible by determining L.D. 50 of a virulent culture in two groups of mice given optimal doses of the vaccine. Two groups of 48 mice were given two injections at weekly interval of 0·0002 ml. each of a casein-hydrolysate cholera vaccine (Haffkine Institute, batch No. 22), and an agar vaccine (King Institute, batch No. 68-P) respectively. One week later, the mice in each group were divided in lots of 8, and given 0.5 ml. of a six-hour peptone water culture of a virulent strain of *V. cholerœ*, type Inaba, in dilutions ranging from 10^{-1} to 10^{-6} in 5% mucin. A group of 40 control mice were similarly divided and given the same doses of the challenge culture. Deaths were recorded upto 72 hours after injection. The results are given in the table below.

It is seen that there is no significant difference in the protective value of agar-grown and casein-hydrolysate vaccines. Mice receiving either of these vaccines withstand approximately 100 times more of the challenge culture than unprotected mice.

Dilution of challenge culture 0·5 ml.	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	L.D$_{50}$.
Casein-hydrolysate vaccine (Haffkine Institute)	0/8	0/8	1/8	5/8	5/8	4/8	..	..	$0{\cdot}5\times10^{-4.53}$
Agar vaccine. (King Institute)	..	1/8	3/8	2/8	5/8	4/8	..	..	$0{\cdot}5\times10^{-4.62}$
Control	..	..	0/6	0/6	0/6	4/8	6/8	6/8	$0{\cdot}5\times10^{-6.44}$

Numerators : Number of survivors.

Denominators : Number of animals used in test.

MALARIA

1. Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.

Work on the preparation of suspensions of DDT and gammexane was continued during the year. After trying a large number of wetting agents it was found that satisfactory suspensions of DDT and gammexane can be prepared by wet grinding the chemical in the presence of gelatine and gum acacia. A suspension concentrate can be prepared in a stone pestle and mortar but with this equipment only ½ lb. of DDT or gammexane can be suitably ground in about half an hour. One labourer working a hand-operated ball mill can grind, in half an hour, 8 lb. to 10 lb. of coarse crystals into a concentrated suspension containing 55 per cent of DDT or gammexane. The concentrate can be diluted to the required strength at the time of use.

Some samples of technically pure DDT cannot be made into a suspension by the above method as crystals instead of being ground into smaller size become coalescent and the whole preparation becomes lumpy. A simple method of preparing a suspension with such samples of DDT has not yet been worked out.

Experience has shown that for indoor residual spraying a stirrup pump fitted with a suitable nozzle is simpler and better than other types of spraying equipment. Neither power sprayers intended for fine atomization of insecticides nor power-spray pumps used for spraying fluid jets or for coarser atomization of insecticides, are suitable for indoor residual spraying against mosquitoes, as their use involves excessive output. The latter can, however, be usefully employed for outdoor spraying against flies. Knapsack sprayers can also be used for outdoor spraying in preference to the stirrup pumps.

In order to carry out comparative tests on various preparations of DDT as an indoor residual spray against adult mosquitoes, field experiments were organized at Delhi and at Gudalur (Nilgiris).

Seven villages were selected in Delhi rural area. In each of these villages 4 catching stations were established and, as in the experiments during the previous year, mosquitoes were collected at regular intervals, both to note the collection per man-hour as well as the total mosquito population in each. Six of these villages were sprayed each with a different preparation, and one was left untreated for comparison. The different preparations used were:—

(1) DDT-gelatine suspension with small quantity of copper sulphate;
(2) DDT-gelatine suspension without the addition of any fungicide;
(3) Geigy's suspension, 50 per cent;
(4) Torch brand DDT emulsion;
(5) DDT-MKE emulsion; and
(6) Gammexane-gelatine suspension.

An analysis of the experiments carried out so far shows that a dosage of 25 mg. of DDT per sq. ft. does not appreciably reduce the prevalence of *A. subpictus* except for about 2 weeks only. *A. culicifacies*, however, appears to be affected by this small dosage for a comparatively longer period. As the number of *A. culicifacies* collected from the comparison village was, however, not very large, no definite conclusion can be drawn from the experiments carried out up to date.

As a result of communal disturbances and extensive flooding of the rural areas, these experiments had to be temporarily discontinued till the middle of October. They are now being continued.

In the Nilgiris, four villages were selected to try different dosage of DDT-gelatine suspension and the usual catching stations were established for making observations as at Delhi. There too, unfortunately owing to the break-down of transport, two of the villages had to be given up. Of the remaining two, one village was treated with DDT suspension, while the other was left untreated for comparison. An analysis of the observations carried out so far shows that with a dosage of 40 mg. per sq. ft. toxicity to *A. fluviatilis* persists beyond a period of 4 weeks. Observations on the prevalence of mosquitoes in the two villages are being continued.

Spreading coefficient of various types of oils was studied with a view to find some adjuvant which would help in spreading a few drops of oil containing DDT on the surface of different types of breeding places of anopheline larvæ. It has been found that the spreading coefficient of different samples of malariol varies between 20 and 30 dynes per c.mm. In one sample, it was in the neighbourhood of 15 dynes only. The addition of certain spreaders increases the spreading coefficient to cover 35 dynes but how these oils will behave on natural waters in the field still remains to be studied. Experiments are being continued.

2. Enquiry on adult *Anopheles stephensi* under the Director, School of Tropical Medicine, Calcutta.

1. *Day-time resting places.*—Owing to the disturbed condition of the city, the search for day-time resting places of *A. stephensi* in houses not under the influence of bovine attraction could not be carried out satisfactorily and had to be abandoned. Nine new resting places, all associated with cows or buffaloes, were, however, discovered. In the case of open cattle-sheds, the resting places were either dwelling or unoccupied outhouses. The new resting places have so far yielded 255 female *A. stephensi*, majority of them engorged or gravid. Unfortunately, the owner of one of the most productive of these places, a buffalo-shed, withdrew permission to collect there after only three visits which had yielded 49 specimens. The total catch of *A. stephensi* during the period under review was 1.205 females and 18 males.

2. *Races of* A. stephensi *in Calcutta.*—Having failed last year to obtain clear evidence of the presence of the *mysorensis* form from the ova laid by wild females, inbreeding experiments were resorted to in the case of doubtful batches of ova and these experiments continued into the present year. Most of such experiments did not result in the recovery of *mysorensis*. One experiment, however, deserves notice here. The ova of the parental generation had the following measurements: total length, 520 microns; breadth, 192 microns; float length, 266 microns; ridge count, 14·6; float length/total length ratio, 0·51. The ridge count and the float length were suggestive of *mysorensis*. Nineteen F1 batches were entirely of type form, the minimum ridge count in any batch being 17. Of 23 F2 batches, 17 were type form and 6 similar to the P generation. 24 out of 25 F3 batches were like the F2 generation. One F2 batch had the following measurements: total length, 508 microns; breadth, 179 microns; float length, 246 microns; ridge count, 13·6; float length/total length, 0·49.

The test of breeding true to *mysorensis* could not be applied to this batch as the ova which were not quite normal in appearance, were infertile. The low measurements, other than the ridge count, can be attributed to imperfect development after oviposition, since it was proved last year that a very significant increase in size occurs after deposition when development is normal. Some of the other F3 batches were inbred to the F5 generation but nothing like that particular F3 batch was encountered. From other wild females 141 batches were also examined this year, but the measurements were not recorded as they were obviously of type form. The conclusion is that var. *mysorensis* is not at present a constituent of the *stephensi* population of Calcutta.

3. *Seasonal prevalence, density, etc.*—Observations on these subjects were continued. The most suitable of the newly discovered resting places are being treated as routine collection stations in order to supplement observations at the few, old stations which have been greatly affected by the disturbances in the city. The routine catches at the old stations have now passed through sixteen months, and while it seems that the monsoon is the season of maximum prevalence of adult *A. stephensi*, rises in numbers were also noted during the spring and autumn. These require confirmation over another year. Adult density was definitely higher this year, and, as detailed below, was associated with the finding of infected specimens. An effort is being made to determine the minimum density for transmission. The composition of the catches in respect of wing and abdominal condition, the latter mainly for new resting places, is being studied. The gonotrophic cycle is being followed both in mosquitoes kept continuously indoors and in mosquitoes kept indoors during daylight hours and outdoors during darkness, as the latter condition appears to correspond more nearly to their natural movements. From May until October the cycle occupied 48 hours in both groups, though temperature conditions were different.

4. *Feeding habits.*—The preparation of anti-sera for precipitin tests has been delayed owing to the disturbances, but the work is now making headway. From material collected last year 259 smears have been tested. The 210 positives included 196 of bovine blood, 9 of both human and bovine blood, and 5 of human blood only. Much material remains to be tested. Smears were also prepared this year in view of the higher adult density. The whole of this work is now being carried out by the staff of the enquiry under the guidance of Lieut.-Colonel S. D. S. Greval, Imperial Serologist, and his assistants.

5. *Infectivity.*—*A. stephensi* numbering 861 were dissected either for gland infection only, or for both gut and gland infection in a smaller number of specimens. Four mosquitoes were found infected: 3 with gland infection and 1 with gut infection only. Two gland infections were in mosquitoes caught in a cow-shed. The first gland infection was obtained in June and the last in October, necessitating revision of previous knowledge as to the transmission season.

6. *Miscellaneous.*— Some laboratory experiments on oviposition habits of *A. stephensi* were performed, and it was found that the species readily laid eggs in tank-water, a situation not apparently favoured under natural conditions. In some experiments, this type of water showed a higher percentage of eggs than tap-water, depending on the position of the bowls containing the two types of water which were provided at the same time. The eggs laid in the tank-water were reared to the adult stage when artificially fed. This raises

the question of *A. stephensi* breeding in the clean tanks of Calcutta in the event of the introduction of a continuous water-supply in which case the cisterns, now the chief breeding places, would no longer be installed. Work on this problem is contemplated for next year.

A few experiments of an exploratory nature to test the influence of bovine attraction on the movements of mosquitoes were carried out with marked mosquitoes. Coloured powders mixed with gum arabic were used for marking. In two experiments with wild-caught *A. stephensi* engorged with blood, the percentages of re-captures after the second day in resting places under the influence of bovine attraction were 16·6 and 12·5 on 18 and 10 mosquitoes, respectively. In two similar experiments with newly bred mosquitoes, the percentages of re-captures were 2·7 and 3·2 on 37 and 31 females, respectively (males were also released). Newly bred mosquitoes appear to have a wider range of dispersal than older mosquitoes. The percentage of re-captures among older mosquitoes must be considered high as only one resting place in a very congested urban area was used as a collection station, suggesting that cattle do exert a strong influence on movements of the mosquitoes, though confirmation is required from an area where this influence is not present. These experiments should be repeated on a larger scale now that several day-time resting places are available for collection of mosquitoes.

7. *Culicoides anophelis.*— Some *A. stephensi* bearing this interesting ectoparasite were again caught this year, and efforts to breed it in order to study its unknown life-history were attended with more success than last year. Material for study of the larval stages has been obtained, but owing to extremely heavy mortality in the early stages, attributed by other workers to cannibalism in the case of other species of *Culicoides*, no pupæ have yet been recovered. It is believed that this difficulty can be overcome with a little more experience in the breeding of the species when it should also be possible to obtain the male, which has not yet been described. Data have been collected on the seasonal prevalence of the parasite, on its effect on the host, and as to whether it does really feed on the blood ingested by the host.

3. Enquiry on the control of sandflies *Phlebotomus argentipes* by spraying with DDT under Dr. D. N. Roy at the School of Tropical Medicine, Calcutta.

The work under review was considerably interrupted on account of disturbed conditions in the city during almost the whole year. It was also extremely difficult to obtain supplies of kerosene oil and sugar, the two important commodities essential for the work. The difficulties of transporting laboratory goods were and still are by no means small.

The work was started in Kaorapukur and in neighbouring villages situated about 11 to 20 miles south of Calcutta. The houses are built mostly of mud walls with thatched roof. The cow-sheds are closely attached to the living quarters. Large number of sandflies are breeding in these cow-sheds also in other localities adjacent to the houses as in rat-holes and in roots of plantain trees. Sandflies are also common in the adjoining villages. The species identified are *P. argentipes, P. papatasi, P. minutus,* and *P. squamipleuris* and according to their density *P. argentipes* stands first.

Precipitin tests have been carried out with the stomach meals of sandflies collected from cow-sheds and living quarters and the sources of blood have been determined in about 1,000 individuals.

The population of sandflies has been found to increase from the later part of May and decrease from the early part of September.

Five percent DDT in kerosene was used for spraying. The actual spraying work was started at the end of May 1947.

The immediate effect of spraying has been the total disappearance of sandflies.

Its residual effect is worth noting. The sprayed places have been free from sandflies for a long period even extending to 3 months. The spraying of a cow-shed with 5% DDT caused a total disappearance of adult sandflies which, however, re-appeared when after 2 days the floor and the walls were smeared with a mixture of mud and cowdung. Thereafter an area measuring 4′×4′ on each wall on 4 sides was sprayed with the result that no adult sandflies have been caught for ± over 2 months. Similarly, the effect of spraying was also observed on mosquitoes which were similarly affected by DDT. Its residual effect was also marked.

Its repellent effect was demonstrated by killing all sandflies in a cow-shed by spraying and thereafter by releasing 150 to 200 freshly caught *P. argentipes* after an interval of a month, when no sandflies were caught after daily search for a week.

Although the results of spraying cow-sheds and living rooms with DDT are encouraging, yet no scientific conclusion can be drawn and further studies will be necessary in order to establish the action of DDT in the eradication of sandflies.

4. Enquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

This enquiry was begun in April 1947, and the proposal for work was chemotherapeutic investigations on (1) Paludrine and (2) CAM-AQ1 in the treatment of malaria.

1. *Paludrine.*— Clinical trials were carried out in a series of 100 indoor patients which included 50 *P. falciparum*, 45 *P. vivax*, 3 *P. malariæ* and 2 mixed *falciparum* and *vivax* infections. Three schedules of treatment were tried: (A) a single dose of 100 mg., (B) a single dose of 300 mg. and (C) 100 mg. thrice daily for 4 days. Children were given proportionately smaller doses. The average period by which the temperature became normal and asexual parasites disappeared from the peripheral blood was 60 hours with (B) and (C) and a little longer with (A). (B) and (C) were found quicker in taking effect and in clearing the blood parasites, the action of (C) being more noticeable than that of (B). Actually the parasites disappeared from the blood within three days—81% in (A), 88% in (B) and 92% in (C). The corresponding figures as regards temperature were 73, 86·5 and 86·5% respectively. In about 2% cases under (B+C) in slightly greater proportion under (A), slight fever or the parasites persisted up to the fifth day, but none had it afterwards. Another feature observed was the relatively quicker parasiticidal action of paludrine on *P. falciparum* than on *P. vivax*.

Relapses.—Of the above 100 patients, 40 were kept under hospital observation from a fortnight to a month; 13 relapsed, viz. 6 out of 8 patients in group A (85·7 per cent), 7 out of 23 patients in group B (30·4 per cent), while the 9 patients in group C remained free. They were all cases infected with *P. falciparum* excepting one of *P. vivax* and one of *P. malariæ*. There were three noteworthy relapses or recrudescences of *falciparum* malaria—one after 4 days of apparent clinical cure with regime A, one after 3 days following regime B and another (not included in the above series) relapsing 9 days after treatment with 100 mg. thrice daily for 10 days. In the latter case the same doses were repeated using a different sample of the drug, but he relapsed again after 11 days when he was given some other anti-malarial drug and this brought down the temperature quickly and he kept well for a month when he was discharged. These results indicate that early relapses of *falciparum* malaria may occur even after 10 days paludrine treatment.

Effect on gametocytes.— Gametocytes were present in the blood of 55 patients before treatment was started and they appeared in the blood of 34 patients during and after the treatment. Paludrine had obviously no action on them. Mosquitoes were fed on a few patients with crescents present in their blood in fair number within a week after treatment, but subsequent dissections of these mosquitoes in the Entomology Department revealed no growth of parasites in them.

Toxic action.—No untoward effect was observed in any patient which could be attributed to the drug. It is worth mentioning, however, that one patient with chronic malarial splenomegaly not included in this series who was having 2 tablets thrice daily complained of severe abdominal pain on the fifth day. He then left the hospital.

Intravenous paludrine.—Paludrine acetate supplied in ampoules 100 mg. each was used in a series of 9 patients including 2 cerebral cases in varying doses with or without oral administration. The results were equally effective, and there was no reaction even after a single injection of the contents of 4 ampoules (400 mg.).

Conclusion.—The small number of cases in this series and the short period of observation would not justify drawing any conclusions beyond stating that the single dose of 100 mg. was unsatisfactory as far too many relapses occurred after it. The single dose of 300 mg. was more promising, but the dosage of 100 mg. t.d.s. for 4 days was more effective than either of the two.

Ten patients, after discharge from the hospital, had a weekly dose of 100 mg. for three to four months, of these two had second attacks during the period. A satisfactory 'complete' paludrine treatment of malaria would ordinarily, therefore, be 100 mg. t.d.s. for 4 days or possibly a single dose of 300 mg. to control an acute attack followed by a bi-weekly dose for six months in order to prevent relapses and incidentally also as a prophylactic. A paper on the subject has been published. The work will be continued to study its effect on a larger number of cases in different doses with special reference to single dose treatment and intravenous therapy to suit rural practice. The results will also be compared with dose of other recent anti-malarials.

2. *CAM-AQI.*—It is a new anti-malarial quinoline derivative which has been shown to be more effective and less toxic than quinacrine in avian malaria, causing no yellow discoloration. It has been tried in 30 indoor cases so far,

using two dosage schemes, viz. (A) 0·1 g. twice daily for 3 days and (B) a single dose of 0·5 g. Twenty-four cases (10 *P. vivax*, 11 *P. falciparum* and 3 mixed *P. vivax* and *P. falciparum*) had regime A, and the period by which the temperature became normal varied from 9 to 42 hours, the average being 24·2 hours, while the asexual parasites disappeared from the peripheral blood from 24 to 72 hours, the average being 48 hours. Only 6 cases (4 *P. vivax*, 1 *P. falciparum* and 1 *P. malariæ*) have so far been treated with single dose of 0·5 g.; the temperature became normal within 18 to 30 hours, the average being 21·2 hours and the blood became negative in 24 to 48 hours, with an average of 38 hours. The patients were kept under observation for an average period of a fortnight in the hospital during which one relapsed, but none had any untoward symptom. The drug neither acted on the crescents nor prevented their appearance. Further observations are in progress.

5. Enquiry into the epidemiology of malaria in the Singur Health Unit with particular reference to the problem of transmission under Drs. R. B. Lal, S. C. Seal and L. M. Bhattacharji at the All-India Institute of Hygiene & Public Health, Calcutta.

Considerable difficulty was experienced in the recruitment of the staff and the collection of equipment and the enquiry could not start functioning till September. We are still short of chemist and other staff. Selection of the area suitable for this investigation presented another difficulty. Barasat, a hyper-endemic area, which lies within convenient distance from Calcutta was originally selected. Being a predominantly Muslim area it had to be given up in view of the tense political situation and uncertainty about its inclusion in one or the other Dominion. After considerable search the present area was selected though it did not satisfy the requirements as well as the previous one (Barasat). It is situated in the main line of the East Indian Railway, about 35 miles from Calcutta in the district of Hooghly and is also approachable by road (Grand Trunk Road).

It was considered desirable that the population under investigation should furnish at least 150 infants and should have a spleen rate of 40% or more. To more or less satisfy these conditions six contiguous villages of the Etachena-Khanyan Union were selected. A map was obtained from the District Health Officer, Hooghly, and enlarged copies were prepared in the Sanitary Engineering Department of the Institute. A preliminary survey was carried out in July-August and location of houses (mosquito-resting places), ponds and other actual or potential breeding places were marked on the map. Schedules giving essential particulars of the breeding places and of each person in the selected population were prepared.

Of the 206 breeding places anopheline larvæ have been collected from 165 places 39 of these are known to breed *A. philippinensis*, 6 regularly and the rest occasionally. Certain characters of these breeding places have been studied. Of the other species of anopheline the following have been found breeding: *A. aconitus*, *A. annularis*, *A. barbirostris*, *A. hyrcanus* var *nigerrimus*, *A. pallidus*, *A. ramsavi*, *A. subpictus*, *A. vagus* and *A. varuna*. *A. hyrcanus* forms 59% of the total catch and *A. philippinensis* only 6·4%.

For adult anopheline collection 274 resting places have been visited. In 26 instances *A. philippinensis* has been found. The adults of all the species

mentioned in the previous paragraph were found but the proportional distribution was not the same. *A. annularis*, *A. hyrcanus* and *A. vagus* were the most common species. *A. philippinensis* proved about 4% of the total catch.

Dissections.—Three hundred and seventy-two mosquitoes composing of *A. annularis*, *A. pallidus*, *A. philippinensis* and *A. varuna* were dissected, none showed instance of infection.

Spleen and parasite.—Only 36 infants have been examined; the parasite rate amounts to 27·8. Attempts are being made to overcome the opposition against examination of infants. Three hundred and sixty-three children have been examined. The spleen and parasite rates are 42·4 and 20·7 respectively.

The species distribution of parasite in blood films so far examined is as follows:—

P. falciparum, 65·8%, *P. vivax*, 30·8%; and *P. malariæ*, 3·4%. One case showed mixed infection of *P. falciparum* and *P. malariæ*. The gametocyte rate of all species amounts to about 6% of the positive films.

Stock solutions for chemical examination of water have been prepared but there being no chemist the chemical work had to be suspended. It is hoped that this work will be started soon after the Puja holidays.

List of papers published.

AFRIDI, M. K., RAMAKRISHNAN, S. P., GOSWAMI, A., and MENON, M. K.—An Enquiry on certain aspects of Paludrine (*I. J. M.*, Vol. I, No. 15).

AFRIDI, M. K., and BHATIA, M. L.—Malaria Control of Villages around Quetta (Baluchistan) with DDT (*I. J. M.*, Vol. II, No. 19).

AFRIDI, M. K.—A Critical Review of the Therapeutic Trials on Paludrine carried out in India (*I. J. M.*, Vol. III, No. 25).

AFRIDI, M. K.—A Scheme for the Control of Malaria in Villages in Delhi Province (*I. J. M.*, Vol. IV, No. 36).

CHAUDHURI, R. N., and RAI CHOWHDURI, M. N.—Clinical Trials of Paludrine, (*Ind. Med. Gaz.*, Vol. 82, No. 5, p. 247).

COVELL, G.—The Malaria Institute of India, 1933-47 (*I. J. M.*, Vol. I, No. 1).

COVELL, G.—A Brief Review of the History and Development of the more important Anti-malaria drugs (*I. J. M.*, Vol. II, No. 16).

COVELL, G.—Bats, Clover, Water lettuce and Malaria (*I. J. M.*, Vol. II, No. 17).

DAKSHINAMURTY, S.—Measurements of Malaria (*I. J. M.*, Vol. II, No. 22).

MULLIGAN, H. W., and SOMMERVILLE, T.—Malaria in the Malabar Squirrel (*I. J. M.*, Vol. II, No. 23).

PURI, I. M., and RAJINDAR PAL.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part I (*I. J. M.*, Vol. I, No. 10).

PURI, I. M., and KRISHNASWAMI, A. K.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part II (*I. J. M.*, Vol. I, No. 11).

PURI, I. M., and BHATIA, M. L.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part III (*I. J. M.*, Vol. I, No. 12).

PURI, I. M., and KRISHNASWAMI, A. K.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part IV (*I. J. M.*, Vol. I No. 13).

PURI, I. M.—The Practical Application of DDT for Malaria Control on Rural and Urban Areas in India (*I. J. M.*, Vol. I, No. 14).

REPORT OF THE MALARIA ADVISORY COMMITTEE.

The Committee met at 10.00 a.m. on 8th December, 1947. It was constituted as :—

Lieut.-Colonel Jaswant Singh. (*Chairman*).
Dr. B. Ananthaswamy Rao.
Rai Bahadur Dr. A. C. Banerjea.
Dr. M. O. T. Iyengar.
Dr. P. Kumaran Nair.
Dr. D. N. Roy.
Dr. D. K. Viswanathan.
Dr. K. S. Viswanathan.
Dr. I. M. Puri.
Dr. S. P. Ramakrishnan. } (*Secretaries*).

The following were also present :—

Dr. A. K. Adhikari.
Dr. A. S. Arora.
Dr. A. C. Chatterji.
Miss M. Craig.
Dr. S. Dakshinamurty.
Dr. B. C. Das Gupta.
Lieut.-Colonel A. N. Duggal.
Lieut.-Colonel B. N. Hajra.
Dr. C. S. P. Hamilton.
Major N. Jungalwalla.
Dr. D. R. Mehta.
Dr. A. K. Mukerji.
Major N. G. S. Raghavan.
Dr. S. Rajendram.
Mr. T. Ramachandra Rao.
Major A. P. Ray.
Prof. K. Subrahmanyan.

Proceedings.

The reports on enquiries carried out during the current year and proposals for 1948-49 were considered.

A. EXISTING ENQUIRIES.

1. *Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.*—Work on the preparation of suspensions of DDT and gammexane was continued during the year. After trying a large number of wetting agents it was found that satisfactory suspensions of DDT and gammexane can be prepared by wet grinding the chemical in the presence of gelatine and gum acacia. A suspension concentrate can be prepared in a stone pestle and mortar but with this equipment only ½ lb. of DDT or gammexane can be suitably ground in about half an hour. One labourer working a hand-operated ball mill can grind, in half an hour, 8 lb. to 10 lb. of coarse crystals into a concentrated suspension containing 55 per cent of DDT or gammexane. The concentrate can be diluted to the required strength at the time of use.

Some samples of technically pure DDT cannot be made into a suspension by the above method as crystals instead of being ground into smaller size become coalescent and the whole preparation becomes lumpy. A simple method of preparing a suspension with such samples of DDT has not yet been worked out.

Experience has shown that for indoor residual spraying a stirrup pump fitted with a suitable nozzle is simpler and better than other types of spraying equipment. Neither power-driven sprayers intended for fine atomization of insecticides nor power-spray pumps used for spraying fluid jets or for coarser atomization of insecticides, are suitable for indoor residual spraying against mosquitoes, chiefly because their use involves excessive output. The latter can, however, be usefully employed for outdoor spraying against flies. Knapsack sprayers can also be used for outdoor spraying in preference to the strirup pumps.

In order to carry out comparative tests on various preparations of DDT as an indoor residual spray against adult mosquitoes, field experiments were organized at Delhi and at Gudalur (Nilgiris).

Seven villages were selected in Delhi rural area. In each of these villages, 4 catching stations were established and, as in the experiments during the previous year, mosquitoes were collected at regular intervals, both to note the collection per man-hour as well as the total mosquito population in each. Six of these villages were sprayed each with a different preparation, and one was left untreated for comparison. The different preparations used were :—

(1) DDT-gelatine suspension with small quantity of copper sulphate ;
(2) DDT-gelatine suspension without the addition of any fungicide ;
(3) Geigy's suspension, 50 per cent ;
(4) Tata's Torch brand DDT emulsion ;
(5) DDT-MKE (Medium kerosene extract) emulsion ; and
(6) Gammexane-gelatine suspension.

An analysis of the experiments carried out so far shows that a dosage of 25 mg. of DDT per sq. ft. does not appreciably reduce the prevalence of *A. subpictus* except for about 2 weeks only. *A. culicifacies*, however, appears to be affected by this small dosage for a comparatively longer period. As the number of *A. culicifacies* collected from the comparison village was, however, not very large, no definite conclusion can be drawn from these experiments.

As a result of communal disturbances and extensive flooding of the rural areas, these experiments had to be temporarily discontinued till the middle of October, but were continued thereafter.

In the Nilgiris, four villages were selected to try different dosage of DDT-gelatine suspension and catching stations were established as at Delhi. Owing to the break-down of transport, observation in two of the villages had to be given up. Of the remaining two, one village was treated with DDT suspension, while the other was left untreated for comparison. An analysis of the observations carried out so far shows that with a dosage of 40 mg. per sq. ft. toxicity to *A. fluviatilis* persists beyond a period of 4 weeks. Observations on the prevalence of mosquitoes in the two villages are being continued.

Spreading coefficient of various types of oils was studied with a view to find some adjuvant which would help in spreading a few drops of oil containing DDT on the surface of different types of breeding place of anopheline larvæ.

It has been found that the spreading coefficient of different samples of malariol varies between 20 and 30 dynes per c.mm. In one sample, it was in the neighbourhood of 15 dynes only. The addition of certain spreaders increases the spreading coefficient to cover 35 dynes but how these oils will behave on natural waters in the field still remains to be studied. Experiments are being continued.

PROPOSALS FOR 1948-49.

(a) Systematic testing of insecticidal sprays, repellents, larvicides and spraying apparatus under field conditions against various vector species in India.

(b) Observations on the bionomic of different vector species in different parts of India.

2. *Enquiry on adult* A. stephensi *under the Director, School of Tropical Medicine, Calcutta.*—Owing to disturbed conditions in Calcutta certain aspects of the work could not be carried out satisfactorily and had to be abandoned.

Observations on the bionomics and different races of *A. stephensi* in Calcutta were continued as during the previous year. A study of egg measurements has so far not revealed presence of the variety *mysorensis* of *A. stephensi* in Calcutta. Maximum prevalence of adult *A. stephensi* was observed during the monsoon, and a slight rise in density also occurred during spring and autumn. These studies require confirmation during the following year. An effort was made to determine the critical density for this mosquito in relation to transmission. A large number of blood meals was collected for precipitin tests.

Infectivity studies on *A. stephensi* are being continued. Out of a total of 861 specimens of *A. stephensi* dissected, 3 showed gland and one gut infection. First gland infection was observed in June and the third in October. A revision of previous knowledge as to the transmission season is required.

Miscellaneous laboratory studies on *A. stephensi* showed that the female laid eggs readily in tank-water, a situation not apparently favoured in nature. In the event of the introduction of continuous water-supply in Calcutta, the possibility of this species breeding in the tanks was considered. On the other hand, the number of cisterns now forming the chief breeding place of this mosquito would decrease.

A few preliminary experiments with marked specimens were carried out to determine the influence of bovine attraction and it appeared that cattle exert a strong influence on the dispersal of *A. stephensi.*

The habits of *Culicoides anophelis*, ectoparasite of *A. stephensi*, were studied.

PROPOSALS FOR 1948-49.

(a) Studies on seasonal prevalence, density and composition of the population of adult *A. stephensi.* Movements of adults in respect of time of exist and entry into resting places, time of feeding, feeding in relation to mating, bovine attraction and dispersal.

(b) Precipitin tests to determine source of blood in different seasons.

(c) Oviposition habits to be studied with special reference to breeding in tanks. Water analysis of the breeding places will be included.

3. *Enquiry on the control of sandflies,* P. argentipes, *by spraying with DDT under Dr. D. N. Roy, Director, School of Tropical Medicine, Calcutta.*— The work under review was considerably interrupted on account of disturbed conditions in the city throughout the year.

Of the five species of Phlebotomus encountered, *P. argentipes* is the most prevalent in Kaorapukur and neighbouring villages situated 1·1 to 20 miles south of Calcutta. The population of sandflies has been found to increase from late May and decreases from early September onwards. Precipitin tests of the blood meal of about 1,000 specimens have been carried out.

Five per cent DDT solution in kerosene was used for spraying and its effect lasted for a period of 3 months resulting in a total disappearance of sandflies from the latter part of May 1947.

PROPOSALS FOR 1948-49.

(a) To conduct experiments on total elimination of sandflies by DDT spraying of all the cattle-sheds in a heavily infested village and to find out the percentage of DDT necessary and its residual effect.

(b) To conduct similar experiments by spraying both the cattle-sheds and living quarters in another neighbouring village under identical conditions.

(c) To test the effect of other insecticides, e.g. bleaching powder, pyrethrum extract, gammexane, copper-sulphate, decoction of tobacco leaves, etc., on sandflies in order to compare their actions with those of DDT.

4. *Enquiry on malaria under Dr. R. N. Chaudhuri, School of Tropical Medicine, Calcutta.*—Clinical trials of paludrine were carried out on 100 inpatients which included 50 *P. falciparum,* 45 *P. vivax* and 3 *P. malariæ* cases. Three schedules of treatment adopted were : (A) a single dose of 100 mg., (B) a single dose of 300 mg. and (C) 100 mg. thrice daily for 4 days. The parasiticidal action of paludrine was found to be relatively quicker in *P. falciparum* than in *P. vivax* infections. The average period by which temperature became normal and asexual parasites disappeared from circulation was 60 hours in schedules (B) and (C) and a little longer in schedule (A),,

Forty patients from amongst the original 100 were kept under observation from a fortnight to a month. The relapse rate was higher in schedule (A) (85·7 per cent) than in schedule (B) (30·4 per cent). The nine cases under schedule (C) did not relapse during the period of observation.

Gametocytes were present in blood of 55 patients before, during and after treatment. However, when mosquitoes were fed on a few patients with crescents and subsequently dissected, no evidence of infection was found.

No toxic action which could be attributed to the drug was observed in any patient. Intravenous injection of paludrine acetate was tried in 100 mg. doses on 9 patients. The results were effective.

It is concluded that the number of cases treated is too small and the period of observation short. However, in the present series of cases it was shown that a single dose of 300 mg. was promising but the dosage of 100 mg. three times daily for 4 days seemed to be better.

CAM-AQI, a new synthetic anti-malarial quinoline derivative, was shown in avian malaria to be more effective and less toxic than quinacrine. The results of preliminary trials on 6 human cases were encouraging.

PROPOSALS FOR 1948-49.

Continuation of chemotherapeutic investigations on the newer synthetic anti-malarials (paludrine, chloroquine and CAM-AQI).

5. *Enquiry into the epidemiology of malaria in Khanyan-Etachena Union (West Bengal) with particular reference to the problem of transmission under Drs. R. B. Lal, S. C. Seal and L. M. Bhattacharji, All-India Institute of Hygiene & Public Health, Calcutta.*— Due to various difficulties, communal and political, a new area 35 miles from Calcutta in the Hooghly district on the E. I. Railway for the investigation was selected and the old one given up. A preliminary survey of the new area was carried out.

The breeding and the daytime resting places of the local mosquitoes were studied, and dissection of 372 mosquitoes comprising *A. annularis*, *A. pallidus*, *A. philippinensis* and *A. varuna* showed no infection.

The spleen and parasites rates were 42·4 and 20·7 per cent respectively, *P. falciparum* predominating *P. vivax* and *P. malariæ*.

PROPOSALS FOR 1948-49.

To continue the enquiry on the same lines as during the previous year.

B. PROPOSALS FOR NEW ENQUIRIES.

1. *Chemotherapy of malaria under Dr. K. Ganapathi, Assistant Director, Haffkine Institute, Bombay.*— Enquiry into the synthesis of compounds of possible value as anti-malarials with particular action on the exo-erythrocytic forms of plasmodia and the testing of the compounds synthesized for their curative and prophylactic effects in *P. gallinaceum* infection in chicks.

2. *Insecticidal enquiry in general with special reference to DDT (chemical and biological) under Dr. D. K. Viswanathan, Assistant Director of Public Health (Malaria), Bombay Presidency.*— Enquiry to determine the para-para isomer content in DDT samples submitted to the Bombay Public Health Department for large-scale use and to assess their efficacy in the field as a residual insecticide with respect to optimum dosage required and the period of residual effect against different species of Anopheles.

The Committee made the following recommendations:—

C. EXISTING ENQUIRIES.

(1) Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi.

(2) Enquiry on adult *A. stephensi* under the Director, School of Tropical Medicine, Calcutta.

(3) Enquiry on the control of sandflies, *P. argentipes*, by spraying with DDT under Dr. D. N. Roy, Director, School of Tropical Medicine, Calcutta.

(4) Enquiry on malaria under Dr. R. N. Chaudhuri, School of Tropical Medicine, Calcutta.

(5) Enquiry into the epidemiology of malaria in Khanyan-Etachena Union (West Bengal) with particular reference to the problem of transmission under Drs. R. B. Lal, S. C. Seal and L. M. Bhattacharji, All-India Institute of Hygiene & Public Health, Calcutta.

The Committee approved the proposals put forward for the continuance of enquiries Nos. 1 and 4 above but did not recommend the continuance of Nos. 2, 3 and 5.

D. PROPOSALS FOR NEW ENQUIRIES.

1. Chemotherapy of malaria under Dr. K. Ganapathi, Assistant Director, Haffkine Institute, Bombay.

The Committee approved this proposal and recommended that investigations be undertaken.

2. Insecticidal enquiry in general with special reference to DDT (chemical and biological) under Dr. D. K. Viswanathan, Assistant Director of Public Health (Malaria), Bombay Presidency.

The proposal for this enquiry was withdrawn by Dr. D. K. Viswanathan.

II. Section 2 (viii) of the minutes of the 7th meeting of the Medical Research Liaison Committee for India, held at the India Office, London, on the 20th March, 1947, was placed before the Committee for perusal.

III. The Chairman informed the Committee that anti-malaria schemes have been initiated for the water-utilization projects sanctioned by the Government of India, e.g. Damodar Valley Project and Hirakud Dam Project on the Mahanadi. The Committee recorded its appreciation.

The Director of Public Health, United Provinces, drew attention to the necessity for the scrutiny of all land reclamation plans in connection with the rehabilitation of refugees, by the Public Health Departments so that the risk of an increase in the incidence of malaria following the introduction of these schemes is averted. Adoption of such a procedure had already proved extremely beneficial in the case of irrigation schemes. The Committee desired to draw attention of the authorities to the importance of introducing a similar system in the current and future reclamation and rehabilitation schemes.

NUTRITION

1. Nutrition researches under Dr. V. N. Patwardhan at the I.R.F.A. Nutrition Research Laboratories, Coonoor.

Work carried out in the Nutrition Research Laboratories during the year has included the following :—

I. Studies on the Vitamins of the B Complex.

Estimation of nicotinic acid in foodstuffs.—A comparative study of extraction of nicotinic acid from foodstuffs with (a) 2 N hydrochloric acid and (b) an enzyme preparation from the pig's intestinal mucosa has been made. The enzyme extraction of pulses yielded higher values of nicotinic acid, whereas the acid extraction of oilseeds and cereals gave the higher values. It is suggested that the discrepancy might be due either to the presence of an alkali-labile precursor of nicotinic acid or a non-specific chromogen. The presence of a precursor has been demonstrated in ground-nut, barley and black gram.

It is suggested that the values for the nicotinic acid obtained by the enzymic extraction of foodstuffs may represent the amount that is biologically available for man.

II. Studies on Hydrogenated Oils.

(a) *Digestibility co-efficient.*—Experiments were carried out on rats with samples of ground-nut oil hydrogenated to different degrees of hardness. These samples melting at 29° C., 37° C., 40° C., 41·5° C., 43·5° C., and 49° C. were kindly supplied free by the Hindustan Vanaspati Manufacturing Co., Ltd., Bombay. The digestibility coefficients of the different fats when fed at 22·8% level were found to vary between 92% and 95%. No correlation between the digestibility and the melting point of fat was found.

Two other samples of hydrogenated ground-nut oil with iso-oleic acid content 9% and 19% respectively, also from the Hindustan Vanaspati Manufacturing Co., Ltd., were compared with ground-nut oil for their digestibility. Using rats as the experimental animals no significant difference between the three could be observed. The values for digestibility coefficient obtained were 94%, 94% and 96%, respectively.

(b) *Growth experiments.*—Five groups of 12 rats each were placed on diets containing the following fats at 8% level : (i) ghee, (ii) purified ground-nut oil, (iii) vanaspati with 19% iso-oleic acids content (vanaspati I), (iv) vanaspati with 9% iso-oleic acids content (vanaspati II), (v) purified coco-nut oil. One group was maintained on a fat-poor diet.

> The animals were four weeks of age at the beginning of the experiment and varied in weight between 40 g. and 50 g. The basal diet consisted of starch 61, skim-milk powder 30, dried brewer's yeast 5, salt mixture 4. In diets containing fat 8 parts of starch were replaced by 8 parts of fat. The animals received in bi-weekly doses 110 I.U. of vitamin A and 70 I.U. of vitamin D per rat per week.

The growth was observed over eight weeks ; on significant difference could be observed between the various groups. The reproduction performance of these animals was then studied. In ghee and coco-nut oil groups 90% mating

were successful, whereas in the remaining groups only 50% were successful. The gestation period was 25 days in the ghee group, 47 days in the ground-nut oil group and 32 days in the coco-nut oil and vanaspati groups.

The tendency on the part of the mother to eat the young was the highest in the pruified ground-nut oil group and none in the ghee group, it was intermediate in the remaining groups of rats. Surprisingly enough no litters of rats on ground-nut oil group survived till the weaning period, all of them being destroyed within a couple of days of birth. The average number of young ones alive on the 30th day per litter was as follows: Ghee 5·5 ; purified coco-nut oil 3·2 ; vanaspati I 1·3 ; vanaspati II 1·1 ; basal diet 0·3 ; and purified ground-nut oil *nil.*

The comparison in the second generation was restricted because the number of animals in each group was not sufficient. It was, however, observed that young ones in all the groups showed hæmorrhages at the angles of the eyes before weaning time. The incidence, however, varied from group to group. On basal diet it was 100%, ghee 12%, coco-nut oil 25%, vanaspati with 19% iso-oleic acids 100% and vanaspati with 9% iso-oleic acids 60%.

In some rats opacity of the cornea of one or both eyes developed at a late stage particularly, in the fat-free group.

The experiments reveal certain peculiar features, e.g. (i) a long gestation period and lack of survival of litters on ground-nut oil, (ii) a slight difference in the reproduction and lactation performance and the incidence of early vitamin A deficiency symptoms between hydrogenated fats containing high and low percentages of iso-oleic acids. These results require confirmation and experiments have already been undertaken for this purpose.

III. The Comparative Nutritive Value of Milk and Curds.

It had been reported by Chitre and Patwardhan (*Curr. Sci.*, December 1945) that the conversion of milk into curds caused certain changes in the concentration of riboflavin and nicotinic acid in the fermented product. A further study was made to find out if any change took place in the carotene and vitamin A concentration during fermentation. Some indication of a decrease in carotene and increase in vitamin A has been obtained. The matter is under investigation.

The biological value of the milk and curds proteins was also tested on rats by the growth method at 8% level of protein intake. The values obtained were 1·30 and 1·28 for milk and curds respectively. The supplementary value of milk and curds to poor rice-diets was also found to be identical.

IV. Studies on Phosphatase.

(a) *Erythrocytes.*— The presence of an alkaline phosphatase in the erythrocytes has been demonstrated. The enzyme has been separated from the acid phosphatase of the erythrocyte which is far more active than the alkaline phosphatase. The latter is weaker than the one in the plasma, and is inhibited by zinc sulphate, sodium cyanide and sodium fluoride, at M/200, M/ 200 and M/50 concentrations, respectively.

(b) *Bone-marrow.*— An alkaline phosphatase from bone-marrow is also under investigation. It is a fairly active enzyme. Its activity is being studied

in relation to age of the animal, the cellularity of the bone-marrow and other conditions that influence the hæmopoietic activity of the marrow.

V. Metabolic Disorders of the Liver.

Certain experiments have been carried out to find out the lipotropic activity of single Indian foodstuffs. A diet with 10% casein and 25% coco-nut oil was used as control, the animals on the diet developed within three weeks fatty livers with 20% crude fat. The whole of casein and part of starch in the diet were then replaced by pulses which in one experiment was extracted with alcohol and in the other not so treated, making the protein level approximate to that of the casein diet. The percentage of liver-fat obtained on these diets was between 9% and 12%. This difference between the liver-fat content on the diets when (a) casein formed the source of protein and (b) when a single pulse formed the source of protein, may probably be due to the difference in the methionine, cystine and choline content of the pulses. An estimation of these three has been undertaken.

An attempt is also being made to study the influence of poor Indian diets on the production of liver cirrhosis in rats. The poor South Indian diet has been taken for experiments with rats as the experimental animals.

VI. Experimental Lathyrism.

The possibility of producing a condition like lathyrism in dogs and monkeys is being explored. Four dogs from the same litter and of the same age were divided into two groups.

> The control dogs had the diet made up of wheat 58, Bengal gram 20, meat 12, oil 5, sodium chloride 2, calcium phosphate 2 and yeast 1 parts. The experimental animals received a diet consisting of *Lathyrus sativus* without husk 80 parts, meat 10, sesame oil 5, sodium chloride 2, calcium phosphate 2 and yeast 1 parts. The records of food-consumption and growth were kept and the appearance of any untoward signs and symptoms in the dogs were also looked for.

Both the dogs on the experimental group showed retarded growth. The gains in weight during five months were for controls 6·58 kg. and 6·65 kg. and experimental 3·09 kg. and 3·38 kg. One of the dogs on Lathyrus diet has developed stiffness of the hind limbs which has affected its stand and its gait. Further signs of neurological involvement are the deviation to the left of the lower jaw and the tongue. Both the ears are constantly held in the erect position. The second dog of the experimental group does not show sign of any disease excepting the retarded growth and a shaggy coat.

A group of 14 monkeys have also been kept on diets containing 88% and 94% of *Lathyrus sativus* with suitable controls. Excepting in one monkey which is showing disinclination to movement, there has yet been no signs of the appearance of the disease in these animals.

VII. Clinical Investigation.

A liaison has been effected with the Government Lawley Hospital, Coonoor, for the study of nutritional diseases, with the permission of the Surgeon-General

with the Government of Madras and the active co-operation of the Civil Surgeon, Coonoor. The Nutrition Research Laboratories now have the facilities for the investigation of out-door and in-door cases of nutritional disorders.

In addition to the above, an Out-patients' Nutrition Clinic has been opened in the premises of the Nutrition Research Laboratories from the 1st of August last. The experience of the last two months has been that there were more cases of vitamin A deficiency than those of other nutritional disorders. The patients frankly suffering from vitamin A deficiency usually came with xerophthalmia, Bitot's spots and follicular hyperkeratosis. There were three cases of advanced keratomalacia as well. The next common deficiency met with was that of the B group of vitamins. Although frank cases of beri-beri were not encountered, peripheral neuropathies were seen. Angular stomatitis and cheilosis were noticed but no case of pellagra has yet been seen.

It appears, therefore, that there must be sufficient material in the Nilgiri hills for purposes of investigation and treatment which can be attracted to the Nutrition Clinic once it gets known widely.

VIII. Investigations on Urinary Calculi.

As a result of the investigations on the subject carried out nearly two decades ago, it was found that diet played a predominant role in the ætiology of 'Stone' in the urinary tract. The wheat-belt in India coincided almost with the Stone-belt. Experimental work on rats further revealed that, amongst the cereals consumed in India, wheat had the highest stone-producing potency, while rice had the lowest, an observation which fitted in with the general picture of stone incidence in India.

Though dietetic habits do not change much in normal times, the abnormally prolonged food crisis through which the country has been passing has brought in its wake drastic changes in the staple article consumed in the different provinces in India. The influence of such changes during a three-year period, 1944-46, on the incidence of the disease is being studied through a questionnaire issued to the major hospitals and heads of medical departments in the various parts of India. The response has been generous.

It is also intended to carry out experimental work both in the rat and in other suitable animals in order to seek confirmation of the results that may be obtained through the questionnaire.

IX. Field Studies.

Soya-bean milk feeding scheme.—(a) The Nutrition Research Laboratories are also co-operating with Dr. S. S. De and Professor V. Subrahmanyan in comparing the influence of cow milk and soya-bean milk on the growth and health of the orphanage children in Bangalore. The report in detail has been given in the note on the scheme submitted by Bangalore workers and hence is not mentioned here.

(b) *Medical evaluation of the nutritional status of the Plantation Labour in South India.*— The Labour Bureau of the Government of India had undertaken an enquiry into the Family Budgets of the Labour of the Tea, Coffee and Rubber Plantations in the country. The enquiry in South India extended over Nilgiris, Shervroy hills, Anamalai hills, Wynaad and Malabar. The

Nutrition Research Laboratories co-operated with the Labour Bureau in assessing the state of nutrition of the labourers and their families in those plantations and evaluating their diets. Owing to the paucity of the staff, however, it was not possible to examine all the families investigated by the Labour Bureau. One estate in each area was selected at random for the nutritional survey. The number of families examined was 136 consisting of 257 adults and 204 children and adolescents.

Among the adults about 80 per cent were subnormal in weight by 10% or more as compared with the Indian averages. Anæmia was widespread. Out of 338 adults and children examined 148 had hæmoglobin less than 10 g. per 100 c.c. Signs of nutritional deficiencies were very common, particularly those of vitamin A and vitamin B complex. A naked-eye inspection could detect advance vitamin A deficiency signs in 25·6% of children and 13·6% of adults. If the criteria of thickening, opacity and localized elevation of the scleral conjunctiva are applied 61·3% of children and 81·2% of adults could be said to suffer from chronic vitamin A deficiency. The evidence of vitamin B_1 and riboflavin deficiencies and paresthesias ascribed to pantothenate deficiency were also met with in both adults and children.

Among other illnesses may be mentioned malaria and vague gastro-intestinal troubles. Ankylostomiasis must be fairly widespread too; out of 52 persons examined in Coorg 21 were found harbouring hook-worms.

X. Nutrition Classes.

Two nutrition classes were held in the year 1947. One was attended by medical graduates deputed by Provinces and States and science graduates who intended taking up nutrition research, the total number being seven. This course lasted from 17th March, 1947 to 30th June, 1947.

A second course was held from the 1st May, 1947 to 30th June, 1947 for laymen such as nursing sisters, domestic science students, health visitors, social welfare workers, etc. The total attendance for this class was 21 comprising of students from all parts of India.

XI. Educational and Advisory Work.

Requests for information and advice were received from all over India including the Central and Provincial government departments, private individuals and firms. The Nutrition Museum in the Laboratories was visited by numerous people.

The Nutrition Research Laboratories also took part in the Food & Nutrition Exhibition organized by the Government of India and held in Delhi in March/April 1947.

2. Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

The biosynthesis of ascorbic acid by the rat and its possible implications.—The problem of the biosynthesis of ascorbic acid by the rat under narcotized condition has been further followed up. It has been observed, as previously reported, that the stimulating effect of chloretone on ascorbic-acid excretion

of the rat can be almost completely suppressed, if the animals are made deficient in either thiamin or riboflavin. The action of chloretone can be restored by correcting the respective deficiencies with thiamine or riboflavin supplement. It has also been observed that chloretone exerts an inhibitory action on the ærobic oxidation of pyruvic acid and the site of action of the narcotic is in all probability a flavoprotein resembling that of Haas cytochrome creductase. The implications of these findings are that both thiamin and riboflavin are involved as direct catalyst in the actual synthesis of ascorbic acid and from the relations of both the narcotic action and thiamin or riboflavin deficiency to pyruvate metabolism, it appears probable that pyruvate or some products of its metabolism is a precursor for the synthesis of vitamin C with thiamin and riboflavin as biocatalysts.

The scheme of work submitted last year included primarily the following aspects of the problems, viz. (i) the *in vivo* synthesis of ascorbic acid in relation to certain factors such as pyruvic acid, thiamin, flavoprotein, narcotic, etc., participating in the process, (ii) the *in vitro* synthesis of ascorbic acid from pyruvic acid by utilizing some chemical reactions involved in the condensation of two molecules of pyruvic acid ($C_3H_4O_3$) to one molecule of the vitamin ($C_6H_8O_6$).

For the study of (i) the Warburg manometric technique was utilized. The tissue was taken from well-fed rats getting a daily supplement of yeast for providing an adequate supply of thiamin and riboflavin to the animals. Liver tissue (about 100 mg.), both minced and sliced, was suspended in Ringer-phosphate (pH 7) in presence or absence of chloretone and shaken in the reaction vessels of the manometer at a constant temperature (37·5°C) with added pyruvate, thiamin and riboflavin. The reaction was followed up both in the ærobic and the anærobic phases for 1½ to 2 hours, after which the ascorbic-acid content of the tissue mixture in the reaction vessels of the manometer was determined by the usual chemical method with 2 : 6-dichlorophenol-indophenol. In some cases certain increase (10 to 30 microgram/gramme) in the ascorbic-acid content of the tissue mixture containing the narcotic was observed over that without the narcotic, in other cases no such difference was found. No conclusive results were, therefore, obtained from these sets of experiments.

Attempts were then made to re-construct in the light of our findings model experiments composed of pyruvate, thiamine-pyrophosphate (co-carboxylase), Haas cytochrome c reductase and chloretone in Ringer-phosphate (pH 7·0) and the artificial system allowed to react in the Warburg manometric vessels at 37·5°C. for 1½ to 2 hours. The results so far obtained are negative.

Co-carboxylase was synthesized according to Tauber (*Jour. Amer. Chem. Soc.*, 1938, **60**, p. 730) and a fair concentrate of cytochrome c reductase was prepared by following the elaborate process of Haas *et al.* (*Jour. Biol. Chem.*, 1940, **136**, p. 747).

The study in more detail of the complicating factors such as oxygen tension, concentration of the phosphate and other electrolytes, concentration of the substrate or other intermediary metabolites and the condition for optimum activity of the prepared enzymes particularly cytochrome c reductase, influencing the process of the synthesis, is in progress.

For the second part of the last year's scheme, viz. the *in vitro* synthesis of ascorbic acid from pyruvic acid by utilizing some organic chemical reactions

attempts were first made for condensing two molecules of pyruvic acid in presence of certain condensing agents such as HCl (gas) pyridine, Na-ethoxide, etc., and the resultant products were analysed for ascorbic acid by the usual chemical method. The utilization of other types of organic reactions involved in the condensation of the molecules of pyruvic acid is also in progress.

It had been previously observed by us (*Science & Culture*, 1943, **9**, p. 564) that irradiation of germinating seeds (*mung* and soya bean) produced an increase in the indophenol reducing power of the seeds. It is proposed to continue the work to find whether this increase is wholly due to ascorbic acid. The effect of B-vitamins on the synthesis of ascorbic acid by germinating seeds will also be studied.

3. Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

With the deterioration of the food situation of the country, a large number of patients are being admitted to the hospital with marked hypoproteinæmia associated with œdema and ascites. It is not always clear from clinical studies whether the low value of serum albumin is due to the defect in the protein synthesis or to protein starvation caused by low protein intake or by faulty assimilation. It seems likely that in many of these patients chronic protein deficiency might have led to pathological changes in the liver resulting in the failure of plasma-protein synthesis.

The effect of high protein diet in the correction of hypoproteinæmia.—Hypoproteinæmia due to protein deficiency can be corrected by a high protein diet provided the patient is in a condition to digest and assimilate the protein. The remarkable success of high intake of certain protein in the prevention of acute necrosis in animals has given rise to the hope that high protein diet may also be of value in correction of hypoproteinæmia due to liver injury.

Our knowledge about dietary treatment is yet scanty. There is evidence that meat and meat extracts may prove positive harmful although opinion is divided. Milk appears to be an ideal diet. Further work on human subjects on the value of different proteins might be well rewarded.

Method of Investigation.

Hypoproteinæmia is not an isolated defect but indicate protein deficiency in general. The level of serum protein alone does not give an accurate idea of the state of the patient during treatment. (1) *Studies on nitrogen balance* and (2) *the level of plasma protein* have been used to determine the effectiveness of the value of the diet in meeting the protein needs of the body.

The excretion of nitrogen in urine by healthy adult on an average Indian diet.—As a preliminary to nitrogen-balance studies on patients, the variations in certain nitrogenous constituents of urine of healthy adults on average vegetarian and non-vegetarian diets were followed. Forty-five observations were made on 12 subjects selected from medical students and members of the staff. A record of food intake was kept. The protein intake in vegetarian diet varied from 40 to 60 grammes, whereas in non-vegetarian diet it varied from 60 to 80 grammes. The variations for 24 hours output are given in Table I :—

TABLE I—A.

Diet	Number of observations	Volume in c.c.	Urea in g. %	Total N2 in g.	Urea N2	NH3
Vegetarian	.. 26	800-1,800	0·4-1·2	4·1-7	3-5·7	0·4-0·7
Non-vegetarian	.. 19	970-1,850	0·9-1·9	6·0-13·9	6-12	0·6-1·2

TABLE—I—B.

The same subject on vegetarian and non-vegetarian diet.

Vegetarian	.. 15	1,375-2,005	0·4-0·9	4·8-7·7	3·5-5·7	0·55
Non-vegetarian	.. 8	1,670-2,000	0·7-1·4	8·2-13·9	7·4-12·0	1·05

The total nitrogen was below 7 g. in 27 of the 45 observations and below 6 g. in 21 instances. Urea below 1 g.% was observed in 50% of the observations.

Creatinine was found to vary within normal range.

The effect of high protein diet on nitrogen balance and the level of plasma protein.—Twelve patients admitted with marked hypoproteinæmia associated with œdema, ascites (or both) were kept on a diet containing 100 g. of protein in the form of milk (60 oz.) with added milk powder and egg. Nitrogen-balance studies were made at different stages. The nitrogen in the fæces was not significantly different from normal showing that the patients had no defect in the absorption of food from the intestinal tract. All patients were in positive nitrogen balance. In spite of positive nitrogen balance the level of plasma proteins were different in different groups of patients.

Six patients with anæmia and œdema due to nutritional deficiency free from any cardiac or renal involvement made uncomplicated recovery during the course of 2 to 4 months. The improvement towards recovery took place in different stages.

(1) There was a gradual increase in the value of r.b.c. and of Hb which was followed by disappearance of œdema and hence a decrease in body-weight. There was no appreciable change in the value of plasma proteins. The general condition of the patient improved with a marked increase in the appetite.

(2) A progressive rise in the level of plasma protein followed closely by an increase in body-weight, the plasma protein returning to normal level. The nitrogen output in the urine showing a gradual increase tending towards nitrogen equilibrium as is shown by case 1. The results of case 1 are shown in Table II—A.

In contrast to the previous cases were a group of 6 patients with ascites and moderate degree of anaemia, clinically declared as cirrhosis of the liver. These patients were treated on similar lines.

With the increase in the value of r.b.c. and Hb, the œdema disappeared. The general condition of the patients improved. The volume of urine excreted

by some of the patients of this group was low (600 c.c. to 700 c.c.). Diuretic had only a transient effect on the excretion of urine. The ascites persisted, but gradually disappeared in all but one. The nitrogen output in urine did not show significant increase as in the previous group. In spite of marked positive nitrogen balance the increase in the level of plasma protein was rather slow as shown by case 11 in Table II—B.

Case 12 made dramatic recovery within 2 months with high protein diet supplemented with casein hydrolysate by mouth, plasma transfusion and methionine. The results are shown in Table II :—

Case 1 : TABLE II—A.

Date	R.b.c. million	Hb.	Total protein	Albumin	Glo-bulin	Weight	Œdema
16–11–1946	1·9	3·7	6·00	3·37	2·63	115	+++++
18–1–1947	5·2	13·5	5·87	3·50	2·37	85	*Nil*
19–2–1947	5·2	13·7	6·37	4·00	2·37	103	*Nil*
30–3–1947	5·2	14·5	7·17	4·70	2·48	105	*Nil*

Nitrogen balance studies during 15 *days before the patient was discharged.*

Nitrogen intake	N2 in fæces g.	N2 in urine g.	Volume c.c.	Date
19 g. (average)	2·7	15·5	2,100	14–3–1947
		15·4	2,000	15–3–1947
		17·1	1,500	17–3–1947
N2 intake—19 g.		18·7	1,830	20–3–1947
N2 in urine 17·4 g.		19·2	2,050	23–3–1947
N2 in fæces 1·7	1	19·0	2,000	24–3–1947
Total 19.1		17·4 average		

The patient was in nitrogen equilibrium when left the hospital. He gained 20 lb. in 2 months after œdema disappeared.

TABLE II—B.

The changes in the value of plasma proteins during recovery.

Date	Total protein	Albumin	Globulin g. %	Eu-globulin	Pseudo-globulin	Weight lb.	Ascites
Case 11.							
11–7–1947	5·93	2·93	3·0	1·68	1·32	138 after tapping	++++
1–8–1947	6·56	3·25	3·31	1·88	1·43	140	+++
18–8–1947	6·69	3·32	3·37	1·81	1·56	—	+
13–10–1947	6·50	3·94	2·56	0·68	1·88	139	*Nil*
Case 12.							
10–9–1947	6·31	2·81	3·50	1·85	16·5	Distension of abdomen.	
22–9–1947	6·87	3·50	3·37	1·25	2·12	Slight	
19–10–1947	7·37	4·19	3·18	1·30	1·88	*Nil*	

Nitrogen excretion in urine.— On a diet of about 20 g. of N2 (125 g. of protein), the total nitrogen in urine increased from 7·68 g. on 25–9–1947 to 15·6 g. on 6–10–47. The patient was still on positive nitrogen balance. He left the hospital to report weekly. The total volume of urine excreted varied from 1,730 c.c. to 2,630 c.c.

Blood volume.—Determination of blood volume along with plasma-protein estimation is of considerable importance in the study of the effect of diet since it helps to give an accurate estimate of the amount of protein formed in the blood during treatment.

In order to standardize the method determination of blood volume was carried out on 12 patients with œdema due to different causes and on 2 normals. The dye method was employed.

4. Nutrition research unit under Dr. H. N. De at the Dacca University, Dacca.

METABOLISM OF FAT.

Effect of coco-nut oil on Ca-metabolism.—Investigation on the deleterious effect of coco-nut oil has been continued. The oil was saponified and the component fatty acids were obtained earlier in several different fractions in the form of methyl esters and the effect of the fractions were investigated on rats by balance studies. Caprylic acid which constitute about 9·5 to 7·8% of the coc-onut oil was found to be definitely deleterious and the rats which were on positive balance on basal diets were all turned negative when the basal diet was supplemented with the fraction containing the said acid (balance with basal diet+6·57 g. with basal diet+caprylic ester, —37·07 mg.). The action of capric acid constituting 7·6 to 4·5 per cent of the coco-nut oil has also been found to be inhibitory on calcium utilization (balance with basal diet+11·6 mg. with basal diet +capric ester—3.95, mg.), The consumption of diet was nearly the same during both the periods of each experiment.

Experiments were also carried out with lauric acid fraction (51·0 to 44·8%) and this was also found to be deterimental to calcium metabolism—the balance of calcuim changed from +106·4 mg. to +73·8 mg. on addition of this fraction. However, the consumption of food by rats with lauric-acid supplement is much less than these when basal diet was given. The fraction containing palmitic, stearic, oleic and linoleic acid esters which together do not constitute more than 15 to 20% of the oil also showed inhibitory effect.

Investigation on the effect of the different portions of this mixed fraction and of the fraction containing myristic acid has been taken to reach a definite conclusion.

The effect of cocogem—a Tata product—on calcium utilization.—The effect of cocogem—a purified product of coco-nut oil on the metabolism of calcium has been studied. The experiments carried on three human subjects have shown that intake of cocogem in place of coco-nut increased the average total elimination of calcium from 605·5 mg. to 676·1 mg. This shows definitely that the purified Tata product, cocogem, is more deleterious than the ordinary bazar coco-nut oil so far its effect on calcium utilization is concerned.

The effect of coco-nut and cocogem on growth and lactation of young rats.— For long-term studies a large number of young rats were divided into three groups—group A, taking ordinary rice diet with milk powder; group B, taking

rice diet with de-fatted milk powder and coco-nut oil; and group C, taking rice diet, de-fatted milk powder and cocogem. They were weighed weekly and after 16th week the average weight of the rats were found to be 202 g. for group A (milk-fat diet), 182 g. for group B (coco-nut oil diet) and 150 g. for group C (cocogem diet). The results indicate clearly that both coco-nut and cocogem are inferior to milk fat as regards their effect on growth. Cocogem was found to be more deleterious than coco-nut oil in this respect.

To observe the effect of coco-nut and cocogem on lactation, the rats of different groups were mated. No litters were obtained in group B (coco-nut oil) and group C (cocogem) even when mated for three weeks. Vitamin E deficiency was suspected in group B and group C of rats and they were divided in two sub-groups and to one sub-group of each group vitamin E was incorporated in the diet and the litters were obtained after some days from both coco-nut and cocogem groups. The litters of cocogem group were all very sickly, while those of coco-nut groups were also inferior to those of the coco-nut oil group. Most of the litters of the cocogem group died on the 2nd to 6th days, while those on coco-nut oil were reared up successfully. The rats of the other sub-groups of group B (coco-nut oil) and group C(cocogem) which did not receive any vitamin E supplement cast no litters. Experiments are being repeated to elucidate this point.

Vitamins.

Interrelation between different factors of B-complex in metabolism.

Effect of graded dose of nicotinic acid on the excretion of riboflavin and nicotinic acid.—Rats fed on diets of low niacin and riboflavin content were daily injected graded dose of niacin ranging from 5 μg. to 1,000 μg. The average urinary excretion of riboflavin was found to be 0·575 μg. and this value rose up to 1·03 μg. when the injected dose of nicotinic acid rose up to 1,000 μg.; but this higher excretion of riboflavin does not seem to be very significant if judged from the view of the high dose of nicotinic-acid injection. The nicotinic-acid estimation of urine revealed that as the injected dose increases from 5 μg. to 1,000 μg. the average retention also increased from 0 μg. to 971·6 μg. This shows that on a restricted diet with low vitamin content the rats possess greater capacity to retain high quantity of nicotinic acid.

The effect of graded dose of riboflavin on the excretion of nicotinic acid and riboflavin.—The average urinary and fæcal elimination of nicotinic acid of rats fed on riboflavin and nicotinic-acid deficient diet were found to be 38·0 μg. and 15·1 μg., respectively and these values did not show any appreciable change when graded doses of riboflavin ranging from 2·5 μg. to 10·0 μg. were injected. This shows that riboflavin do not possess any effect in the metabolism of niacin.

The effect of injecting graded dose of riboflavin on its retention showed that the urinary excretion of 0·82 μg. of riboflavin on basal diet rose progressively up to 4·3 μg. as the injected dose of riboflavin rose up to 10 μg. In every dose a definite relation between the dose and the retention was observed and it was found that at all levels of injected dose the percentage of retention remained constant at 30 per cent. This seems to show that the rats possess capacity to retain not more than 30 per cent of the injected dose of riboflavin. The fæcal excretion of riboflavin did not show any change after the injection of the graded doses of this vitamin.

The effect of sulpha drug on the excretion of B-vitamins.

Rat experiments.— The effect of sulpha drugs on the biosynthesis of different factors of B-complex has been taken up this year. In the present investigation sulphamezathine (sulphadimethylpyrimidine of Imp. Chem. Phar., Ltd.) has been chosen for its lower toxicity. Rats kept on basal diet with very low niacin and riboflavin content were divided into two groups, one taking the basal diet and the other basal diet *plus* sulphamezathine at a 2 per cent level of the diet. It was observed surprisingly that the total urinary and fæcal excretion of riboflavin and niacin of rats on the above sulpha-drug supplement was much higher than those of rats on basal diet alone. The increased excretion was found to be more significant in urine than in fæces. The average daily urinary excretion of riboflavin and niacin by the rats kept on basal diet were found to be 0·83 μg. and 38·0 μg., respectively, while on sulpha-drug supplement they were found to be 2·6 μg. and 67·8 μg., respectively. As the diet was almost deficient in niacin and riboflavin, the possibility of the increased excretion of the above two factors on sulpha-drug supplement as due to increased absorption of dietary factors has, therefore, been ruled out that this increased urinary drainage of riboflavin on sulpha-drug supplement was not from the body-store was revealed by the fact that when graded doses of riboflavin was injected to rats on sulpha-drug supplements they retained a considerable amount of the injected dose. Moreover, the percentage retention of the injected dose remained always constant at 30 per cent at all doses even when the rats were given sulpha-drug supplement. Further experiments are being continued to elucidate the reason of the increased excretion of niacin and riboflavin on the supplement of sulphamezathine.

Human experiments.—Experiments carried on four human subjects with sulphadimethylpyrimidine have shown that when the above drug in 3-g. dose was given daily the average daily urinary excretion of nicotinic acid rose from 20·5 mg. to 41·4 mg. This is quite in good agreement with the results obtained by rat experiments. The riboflavin estimation of the human urines on sulpha drugs is being made.

The effect of this drug on the vitamin B_1 excretion produced quite opposite results. It depressed the thiamine excretion to an appreciable extent from 118 μg. to 25·2 μg. on average. It has definite inhibitory effect on the growth of the intestinal flora synthesizing vitamin B_1.

The lower excretion of thiamine in urine by sulpha does not seem to be due to destruction of this vitamin by the drug when 9 mg. of thiamine were fed to the human subjects along with sulpha drug a considerable amount of the above vitamin was retained. About 95 per cent of the ingested thiamine was retained even when given along with the above sulpha drug.

This experiment opened a new line of approach for the study of the human requirement of thiamine and other factors of B-complex—after inhibiting the intestinal synthesis of the above vitamins by well-selected sulpha drug, which has so long been overlooked for the assessment of human requirement. Experiments have already been begun in this line.

Ascorbic-acid requirment for saturation.—A study of the ascorbic-acid requirements for saturation of adult human subjects has been undertaken this year. Experiments were conducted on five hospital patients kept under proper care and consuming non-vegetarian rice diet.

In the present investigation the whole experimental period was divided into two parts, viz. (1) saturation period and (2) experimental level period according to the technique followed by Kline and Heart (*Jour. Nutr.*, 1944, **26**, p. 413).

Each experimental period consisted of a 5-day saturation period followed by 6 days on the experimental level period. During the saturation period 200 mg. of crystalline ascorbic acid was given to each subject for 4 days. On the fifth day a 400 mg. test dose was given and if 50% or more of the total intake for that day was excreted in 24 hours, the subject was considered to be saturated. After the subject has been saturated they were fed with the same controlled diet and the experimental level period with graded doses of ascorbic acid was begun. This was continued for 6 days. On the seventh day the 400 mg. test dose was again given and if 50% of the 400 mg. appeared in the urine, it was considered that the level of the previous 6 days intake had been sufficient for the subjects' saturation needs.

By this procedure it was found that one subject became saturated at 75 mg. level of intake, another at 85 mg. of intake and the other three between 85 mg. and 100 mg. of intake.

Availability of vitamin C of lemon (Jambura) by adult human.—Much work has been done on the vitamin C content of the various citrus fruits in India but there is no literature regarding the availability of this vitamin of these fruits. Work has, therefore, been taken up in this line according to the technique proposed by Clayton and Borden (*Jour. Nutr.*, 1943, **25**, p. 349) and Hertzler (*Jour. Nutr.*, 1945, **30**, p. 355). The fruit chosen was lemon (*Jambura*) which is very cheap and contains a fairly large amount of ascorbic acid (45 mg. to 60 mg. per 100 c.c. juice). The experiments were conducted on three college hostel students of 20 to 25 years of age. The experimental periods were (1) adjustment period for 3 days with 300 mg. of pure ascorbic acid (Redoxen), (2) saturation period for 7 days with 200 mg. of vitamin C, (3) control period for 7 days with 75 mg. of pure ascorbic acid, (4) saturation period for 7 days again with 200 mg. of ascorbic acid and (5) experimental period for 7 days with 25 mg. pure vitamin C *plus* the amount of *Jambura* juice needed to provide 50 mg. of vitamin C.

Comparison of the urinary elimination of vitamin C of the periods 3 and 5 have shown that the ascorbic acid of *Jambura* fruit is as well utilized as pure ascorbic acid and there is no enzymatic destruction in the body. The average daily urinary excretion of ascorbic acid in control period (period 3) was found to be 44·4 mg., while in experimental period (period 5) the value was 45·5 mg.

Nicotinic-acid study on malnutrition patients.—Experiments were conducted on five malnutrition patients who took restricted ration of the destitute home. The diet contain 5 mg. to 8 mg. of nicotinic acid. The average elimination on these diets ranged from 3 mg. to 7 mg. To test whether the nicotinic acid of the diet was sufficient for these malnutrition patients, the technique of Sarett *et al.* (*Jour. Nutr.*, 1945, **29**, p. 335) was adopted. In his method if after test dose of 500 mg. of nicotinic acid, the extra excretion did not exceed more than 5 per cent of the test dose, the dietary nicotinic acid was regarded as sufficient. In our case the dose was gradually increased from 100 mg. to 500 mg. At 100-mg. dose the extra excretion exceeded 20 per cent and at 500-mg. dose this extra excretion came down to 10 per cent level. This shows that the patients were highly malnutritious and 5 mg. to 8 mg. of nicotinic acid of the diet was not sufficient

to make them saturated. The authorities have been instructed to give extra dose of pure nicotinic acid to these patients.

Vitamin B_1 content of some indigenous drugs.— It was felt interesting to enquire into the B_1 contents of indigenous drugs used in the treatment of beri-beri. According to the Ayurvedic mode of treatment some "Aristas" are used in cases of dropsy and beri-beri. "Aristas" are generally prepared as follows:—

Decoction is made of indigenous drugs by boiling in water. To this decoction is added old molasses. It is kept there in closed earthen jars and allowed to ferment by the wild yeasts and other organisms present in the molasses. After few months the jars are opened, the liquid is filtered and is ready for use.

B_1 content of some "Aristas" as 'Asokarista'. 'Balarista', 'Draksharista', etc., were found to range between 18 μg. and 49 μg.

Mineral Metabolism.

Food value of whey.—Whey is a bye-product of casein prepared mainly for the purpose of sweetmeat preparation in our country. This is thrown away as waste. The nutritional value of whey, being a milk-product, was considered to be of some importance, especially in respect of calcium which is very much deficient in Bengali diet.

Experiments on both rats and on human subjects were undertaken. The results on different human subjects proved to be of much importance. Supplementation of 350 c.c. of whey to the diet of the subject supplied about 130 mg. to 190 mg. of extra calcium and about 80 to 88 per cent of this whey calcium was utilized. All the items of the diet were prepared by whey. The experiments on rats also proved to be very significant. The percentage utilization in this case was found to be 85.

The determination of biological value of whey protein was also undertaken but the rats refused to take the food at the 5% level of dried whey. Consequently the experiment was discarded.

It may fairly be concluded that whey, a waste product of sweetmeat-makers has got a very important food value. Whey, if taken daily with the poor rice diet, may compensate to some extent the calcium deficiency of Bengali diet.

Phytic acid.—Investigation on metabolism of phytic acid on three human subjects had been carried out. It was found that about 57 to 65 per cent of total phosphorus of food is taken as phytin phosphorus. Of this phytin phosphorus, 45 to 50 per cent is recovered unchanged in the fæces. The remaining 50 to 55 per cent is supposed to be metabolized in the human system.

Lathyrism.

In continuation of the work reported last year, different experimental animals, e.g. chicken, pigeons and guinea-pigs, were fed with *Lathyrus sativus* at 30 per cent level of food-intake, feeding Bengal gram as control. The experiment was continued for 5 weeks and the injurious effect of *Lathyrus sativus* on growth and general physical condition of the animals was observed.

Attention was directed to isolate the toxic principle responsible for the development of lathyrism, present in *Lathyrus sativus*. Finely powdered *L. sativus* was, therefore, extracted completely with petrol-ether (50°C. to 65°C.) and the oil (1·22 per cent of the seed) and the extracted seed were fed to guinea-pigs separately. The oil was diluted ten times with ground-nut oil.

Guinea-pigs weighing between 145 mg. and 165 mg. were uniformly devided into four groups and fed respectively with the four diets: (1) normal diet containing Bengal gram (group A), (2) with Bengal gram and *L. sativus* oil (group B), (3) with *L. sativus* (group C) and (4) with extracted *L. sativus* (group D).

Animals of group A showed excellent growth and general good health.

Animals of group B showed retardation of growth after 3rd week, general weakness and reluctance to move about. They all died on the 6th week.

Some animals of group C showed definite paralysis of hind legs, besides general ill-health at the end of 5th week and died after two days.

Animals of group D showed also slight retardation of growth, loss of hair and reluctance to food-intake. They died in the 8th week.

No definite conclusion could be drawn from this preliminary experiment and further work is being continued.

Soya Bean.

Last year's work on soya bean was continued. Biological value of soya-bean milk protein was determined by balance-sheet method on rats.

Soya-bean milk powder was prepared as follows:—

Soya bean previously soaked in water for 24 hours was heated with water after addition of a few c.c. of glycerine for about one hour. It was then thoroughly washed to get rid of the seed coat and made into a fine paste in a stone mortar with minimum amount of water. This was mixed thoroughly with four times its volume of water, boiled and filtered through muslin.

The filtrate was then evaporated to dryness under reduced pressure. The residue was then finely powdered. The average composition of the soya-milk powder thus obtained is as follows:—

	Per cent.
Protein	41·70
Fat	33·45
Carbohydrate	18·42
Ash	4·37

The biological value and the digestibility of the soya-bean milk protein at 5 per cent level of intake was found to be 69·7 and 89·2 per cent, respectively.

Nutrition Exhibition.

This year in January a Nutrition Exhibition was organized in this Laboratory through the joint co-operation of the workers of the Nutrition Research Unit and the research students of the University on the foundation day of the University. About ten thousand visitors attended the exhibition.

Lectures on nutrition.—Three nutrition lectures by slides have been organized by the workers of this Unit in three local schools. More invitations

to deliver some more lectures in schools and colleges at Dacca have yet to be complied with.

5. Nutrition research unit under Dr. R. G. Chitra at the Seth G. S. Medical College, Bombay.

I. *Fat metabolism.*

Absorption of fats from human intestine.—The comparative rates of absorption of butter, coco-nut oil, ground-nut oil and its hydrogenated product from the human intestines, have already been worked out. The aim of the present investigation is to find out the effect of certain dietary factors on the rate of absorption of various edible fats. Use has been made of the Frazer's chylomicron technique.

(A) *Effect of carbohydrate.*—Monosaccharides have been found to interfere with the absorption of butter from human intestines, while diasaccharides had no such effect. Some figures which elucidate this point are given in Table I :—

TABLE I.

Effect of some carbohydrates on fat absorption by chylomicron count.

Time in hours		1 oz. butter +1 oz. cane-sugar	1 oz. butter +1 oz. lactose	1 oz. butter +1 oz. glucose	1 oz. butter + 1 oz. fructose
Fasting	0	1	2	2	3
	1	17	8	9	6
	2	15	24	10	10
	3	8	53	7	33
	4	7	23	9	4
	5	..	16	15	14

It is probable that the phosphorylation of fat may be delayed by the presence of a monosaccharide which also requires a phosphate radical for its passage through the epithelial cells. It has, therefore, been proposed to conduct experiments to verify this presumption.

It is also intended to investigate the effect of certain members of B-complex, essential fatty acids and lower saturated fatty acids on the rate of absorption of fats. The chylomicron technique has been successfully applied to rats, a fact which has facilitated the working out of the absorption rate of larger number of fats particularly those which are unpalatable to the human beings in a pure form.

(B) *Comparative nutritive values of dalda, vegetable oil and butter.*—As regards the nutritive value of butter, vegetable oils or hydrogenated oils there exists a wide difference of opinion. Some workers have found that there is no significant difference in the absorption and utilization, while others hold that certain hydrogenated oils are injurious to the health if fed for a sufficient length of time. Majority of the investigations are done using synthetic diets as basal diets. The effect of these fats would, however, be better appreciated from the point of nutrition of the population if experiments are done using regional diets as the basal diets. Work has, therefore, been undertaken to study the compara-

tive value using a diet of a poor class of population of the Province. The diet which is otherwise balanced as regards proximate principles was selected from the results of diet surveys carried out at this College some time ago (Nigoyi *et al.*, 1939).

The diets were prepared freshly every alternate days. The fat constituted 9 per cent of the total diet. In addition to this the nutritive value of a high fat diet (containing 23 per cent of fat) which diet also was based from diet surveys in another section of community is under investigation. This diet is purely vegetarian and contains a larger quantity of milk.

The results so far obtained have tentatively shown that there was no significant difference in the growth rates of the animals on the diets containing 9 per cent fat. As regards the diets containing high fats per cent (23 per cent) the ground-nut oil group showed lesser growth. In reproductive capacity none of the diets containing 9% fat showed encouraging results. The work is in progress on subsequent generations.

2. *Nutritive value of pure-bred strains of cereals and pulses.*

In a previous report it has been stated that the study of the environmental conditions and genetic influence on the nutritive value of cereals and pulses would be a step forward towards the development of the nutrition of this country and on that consideration it was proposed to undertake a systematic work to study the genetic influence on the nutritive value. For that purpose the Director of Agriculture, Bombay Presidency was requested to give necessary assistance at their agricultural farms but since the necessary co-operation was not forthcoming the problem had to be deferred to more opportunate times. The results of previous work have been sent for publication.

3. *A search for carotonic rich foodstuffs of vegetable orijin.*

In last year's report it was stated that experiments are being conducted to study the stability of carotene. For this purpose the carotene content of orange peels, citrus peels and lemon peels has been studied at various stages when pickles were prepared from them. The results have shown that usual colorimetric procedure which is employed to estimate the carotene contents shows a rise in the value of carotene on preservation but chromatogram showed that the increase in value was not due to carotenes but to some other colouring matter. Other experiments to study the stability are in progress. Results of previous findings have been published.

4. *Studies in nicotinic acid.*

(A) *Enzymic hydrolysis.*—It has been found that values of nicotinic acid on foodstuffs (cereals and pulses) obtained by chemical methods are high and give but a rough idea as to the exact amount of the vitamin available for human or animal nutrition. Even microbiological methods evolved recently have helped to solve this problem only in a partial way. Attempts have, therefore, been made in this Laboratory to evolve a technique making use of the enzymes which are usually involved in the normal process of digestion. Enzyme preparations used were from rats stomach and intestinal mucosa, since the rat was thought to be a easily available animal in a nutritional laboratory. The cereals and pulses were incubated successively with the enzyme preparations

and the nicotinic acid thus liberated was estimated colometrically. It has been found that the values for nicotinic acid obtained by this method were invariably lower than those obtained by the usual chemical methods. Some suggestive figures are shown in Table II :—

TABLE II.

Nicotinic-acid content by enzymic hydrolysis of cereals and liver (in μg. per g. of the cereal).

	Acid hydrolysis.	Enzyme hydrolysis.
Rice	106·8	38·8
	107·1	40·8
	107·6	35·2
	107·3	37·1
Wheat	111·3	39·35
	107·0	38·95
	108·1	40·3
	102·6	38·0
Liver of freshly killed adult rat	132·8	107·2
	131·0	110·0
	136·6	108·2
	133·7	108·9

The findings suggest that probably all the nicotinic acid as determined by acid hydrolysis—from vegetable foodstuffs is not set free during the process of digestion and such case may not be available for the nutritional needs.

So far only rice and wheat have been investigated and further work on few other cereals and pulses is in progress.

Experiments *in vivo* are also being simultaneously conducted to correlate these findings.

(B) *Studies on estimate of nicotinic acid from vegetable foodstuff.*— Experiments were conducted to evolve a suitable chemical method for the estimation of nicotinic acid from vegetable foodstuffs and if possible to differentiate the same from its so called precursor. The following sets of experiments were carried out :—

(1) Acid extraction of food material which was boiled and subsequently treated with acids or alkalis of different strength.

(2) Acid incubation of food material and subsequent treatment with acids or alkalis of different strength.

(3) Extraction with buffers of different pH. Some representative figures are shown in the Table III :—

TABLE III.

Nicotinic acid in μg. per cent in wheat flour after incubation with buffers of different pH.

Time in hours.	1 pH.	7 pH.	10 pH.
1	3,718	3,581	3,740
3	3,637	3,599	3,804
4	3,906	3,825	4,700
6	4,400	4,546	4,975
16	3,827	4,535	4,763
24	3,207	4,224	4,213

The last method was found to be most satisfactory and was applied to few cereals and pulses. The work is in progress. This technique is utilized to differentiate between the free-nicotinic acid and its precursor which according to Elvehjen *et al.* occurs in vegetable foodstuffs in a considerable amount.

5. *Researches on vitamin D and phosphatases of bone and tissues.*

In an earlier communication (Dikshit and Patwardhan, 1947) it was stated that in albino rats the serum-alkaline phosphatase value showed a gradual fall in normal as well as severely rachitic animals, thus throwing a doubt on the osseous origin of increased serum phosphatase which was observed to be a feature in rickets.

To determine whether the bone and serum phosphatases were same in bone diseases like rickets, experiments were carried out to observe the activity of the two enzymes in different pH media and various substrates. In other set of experiments the effect of Mg ions on the two phosphatases was also studied.

The tentative conclusions drawn from these studies are as follows:—

(i) Both the serum and bone phosphatases have an optimum pH range of 8·9, 9·2. At pH 7 and 5, the activity of both the enzymes is considerably reduced. At pH 5, the activity is less than at pH 7.

(ii) The presence of Mg ions enhances considerably the bone-alkaline phosphatase activity, whereas only a slight effect is produced on serum-alkaline phosphatase both in normal and rachitic rats.

(iii) From the experimental data obtained up to date it appears that there is a greater fall in serum-alkaline phosphatase level in rachitic than in normal animals, while at the same time the bone-alkaline phosphatase level is at a higher level more so in former than in the latter.

Conclusions (ii) and (iii) support, if at all, the doubt expressed in the earlier communication.

Work is also in hand to find out whether Gomori's technique would be helpful in corroborating the above findings.

6. Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

Biological availability of iron in the whole and the lecitho-vitellin protein fraction of egg-yolk (duck).— The nature of iron in the whole egg-yolk and the absorption of added metal ions, by the whole egg-yolk or by lecitho-vitellin fraction have been previously reported. In order to see whether the iron present in egg-yolk or lecitho-vitellin faction is biologically potent, and whether protein of egg-yolk has any augmenting effect on the absorption of iron the following lines of investigation have been carried out.

It has been observed by Sherman *et al.* (*Jour. Biol. Chem.*, 1934, **107**, p. 289) that though egg-yolk contains ionizable iron, it is apparently not fully utilized in hæmoglobin regeneration due to the fixating action of sulphur of the egg-yolk on copper which helps mobilization of iron required for hæmoglobin regeneration. In order to see whether copper plays its role in the hæmoglobin regeneration by helping the process of hæmopoiesis from absorbed iron or simply by enhancing absorption of iron, the following feeding and balance-sheet

experiments were carried out with rats fed with supplements of the following substances as the source of iron : (i) ferric chloride, (ii) egg-yolk dried at 80° C., (iii) lecitho-vitellin protein prepared from egg-yolk, (iv) egg-yolk enriched with added iron (dried at 80° C.), (v) lecitho-vitellin protein enriched with added iron. Conditions of experiments were as follows :—

> Average weight of rat=53 g. ; diet is milk : period of observation—15 days ; as source of iron the amount of egg supplement or ferric chloride supplied was so adjusted that 1·0 mg. of iron was given per rat per day. 0·05 mg. copper as copper-sulphate solution was supplied per rat per day along with milk ; in one group with egg-yolk as source of iron, copper supplement was omitted to see the effect of copper on the absorption of iron.

Statistically considered, the results obtained so far indicate that percentage of retention of iron whether it comes from egg-yolk or from lecitho-vitellin is of the same order as that of ferric chloride (percentage of iron retained in the body is 68 to 72 in case of ferric chloride ; with egg-yolk and lecitho-vitellin it is 74 to 81 and 71 to 76, respectively). The results further seem to show that copper has also no significant effect on the retention of iron by the body. These results also indicate that so far as iron absorption and retention in the liver of rats is concerned iron of egg-yolk is quite comparable to the iron of ferric chloride, though according to Sherman in hæmopoiesis the iron of egg-yolk is less effecive than the iron from ferric chloride when given without any supplement of copper. The function of copper appears to be to help hæmopoiesis directly and not indirectly by inducing a greater absorption and retention of food iron.

Iron and phosphorus of cereals and pulses.—McCance and Widdowson (*Biochem. Jour.*, 1935, **29**, p. 2694) have shown that ferric phytate is very insoluble at an acidity corresponding to that which prevails in the stomach and in the small intestine. It seems, therefore, highly probable that ferric phytate is a poor source of iron for nutrition. The implications of this have a special bearing on the utilization of iron of cereals, as a considerable part of the phosphorus of cereals is in the form of the phytate of iron and other metals. So this may affect the availability of iron present in cereals. Some chemical analysis of a few samples of cereal grains and pulses was carried out to determine their total iron, total phosphorus and phytic-phosphorus content.

Figures for total iron content (mg./100 g. dry basis) vary between 6·8 and 26·0. Figures for total phosphorus content (mg./100 g. dry basis) vary between 146 and 285 ; those for phytic phosphorus between 33 and 140.

As all these foodstuffs are taken in fully boiled condition and as phytic phosphorus increases with boiling of cereal grains (Giri, *Ind. Jour. Med. Res.*, 1938, **25**, p. 869), two samples (rice and lentil) were analysed for phytic phosphorus after boiling for ½ hour. It has been observed that in case of rice, percentage of phytic phosphorus rises from 140 to 202 and in case of lentil, from 100 to 123. This released phosphorus may indirectly influence the availability of iron.

Biological availability of iron of cereals.—Mere addition of cereal food as a source of iron does not induce normal hæmoglobin formation (Elvehjem, *Jour. Biol. Chem.*, 1934, **107**, p. 383) in anæmic rats. Cereal grains and pulses contain a high amount of phytic phosphorus (which again increases on boiling) and this phytic phosphorus of cereal grains interferes with the absorption of

iron by combining with it. In a more recent piece of work it has been observed by Fuhr and Steenbock (*Jour. Biol. Chem.*, 1943, **147**, p. 59) that with a synthetic ration furnishing a limited amount of iron as ferric chloride and an amount of calcium optimum for growth and calcification, slightly less hæmoglobin was produced in rats when optimum amount of phosphorus was supplied as phytic acid rather than as a mixture of mono- and di-potassium phosphate; with milk supplemented with copper as the basal ration, a limited amount of iron as ferric phytate produced less hæmoglobin than an equal amount of iron in the form of ferri-alum. In order to see whether the reduced hæmoglobin regeneration with cereal grains and ferric phytate as source of iron is due to poor absorption of iron from these sources experiments were carried out with rice (boiled) and lentil (boiled) as the sole source of iron. The amounts of rice and lentil supplied were so adjusted that each rat got 0·5 mg. iron per day. In addition to the cereal and pulse, 0·1 mg. of copper as copper sulphate solution was given daily to each rat. Apart from these, calcium and phosphorus were added to the diet during cooking, so that it contained an optimum amount of calcium and phosphorus. Calcium was supplied as calcium carbonate and phosphorus as KH_2PO_4. Milk was supplied *ad lib.* To see the effect of copper on the retention of iron from rice or lentil, groups of rats were run without copper supplement.

From the results obtained it appears that—

(i) Iron of both rice and pulse (lentil) are poorly absorbed in comparison with ferric chloride and the percentages of absorption with these two diets are of the same order as that with ferric phytate (percentage of iron retained in case of rice varies between 24 and 38; those with lentil and pheric phytate are between 34 and 42, and between 28 and 42, respectively).

(ii) Added iron to rice and pulse during boiling is also rendered biologically unavailable (percentage of iron retained in such groups varies between 20 and 34).

(iii) Copper has no effect on the absorption of iron from rice and pulse.

7. Enquiry on poor rice diets and food preparations in common use in South India under Dr. Eleanor D. Mason at the Women's Christian College, Madras.

I. *Analysis of South Indian food preparations.*—The analyses of green leafy vegetables used by the common people in Madras city, partially reported in the 1946 I.R.F.A. Report, have been completed. All greens with the exception of Paruppu keerai and radish tops were collected from among the weeds in the college compound, the two samples being collected from different parts of the compound. Two analyses were made of every sample. Only edible portions were used—thorns in Nerringi and flowers and pods of Vella keerai and flowers of Kuppameni being discarded. The means and the range of two samples are shown in Table I.

TABLE I.

Analysis of green leafy vegetables used by the common people in Madras city.

Tamil name.	Botanical name.		Moisture, g.	Protein, g.	Ether* extractive, g.	Total Ash, g.	Calcium, g.	Phosphorus, g.	Iron, mg.	Calories.	Vitamin C, mg.
Paruppu keerai	*Portulaca oleracea*	Mean	94·11	1·67	..	2·408	·084	·051	25·09	35	26·03
		Range	93·08	1·4	..	2·33	·072	·049	21·95	33—38	..
			94·38	1·98	..	2·96	·094	·054	28·36	..	..
Radish tops	*Raphanus sativus*	Mean	90·37	2·43	..	2·295	·289	·039	17·83	46	119·5
		Range	90·03	2·04	..	1·78	·286	·039	15·27	31—60	124·3
			90·71	2·83	..	2·81	·291	..	20·4	..	109·8
Kuppameni	*Acalypha indica*	Mean	80·54	6·74	1·41	3·14	·667	·099	17·31	64	147·9
		Range	75·81	6·58	1·27	3·31	·68	·05	16·97	63—66	147
			85·25	6·91	1·55	2·96	·57	·14	17·65	..	157
Mukarratai	*Boerhavia repens*	Mean	84·53	6·09	·90	4·31	·667	·099	18·39	65	27·23
		Range	82·56	5·44	·71	3·60	·65	·10	18·31	59—72	31·03
			86·48	6·75	1·09	4·40	·68	..	18·47	..	23·43
Pacharisi keerai	*Euphorbia hirta*	Mean	78·14	4·65	1·71	3·15	·546	·106	21·24	120	44·32
		Range	77·66	4·31	1·72	2·77	·46	·07	20·44	116—113	..
			78·62	5·07	..	3·54	·63	·14	22·04	..	..
Vella keerai	*Cleome viscosa*	Mean	80·41	5·64	1·85	3·75	·881	·073	24·45	93	203·6†
		Range	76·23	5·09	1·67	3·31	·78	·07	22·95	83—105	..
			84·61	6·21	2·04	4·20	·98	·09	..	..	..
Nerringi	*Tribulus terrestris*	Mean	79·09	7·81	·541	4·37	1·55	·082	9·22	94	41·53
		Range	78·99	6·83	·46	4·54	1·40	·07	8·79	85·5	40·88
			79·19	7·62	·62	4·72	1·72	·09	9·66	91·5	42·18
Saravallai keerai	*Trianthema monogyna*	Mean	91·33	2·09	·390	2·62	·056	·03	38·54	36	69·60†
		Range	91·10	1·75	·33	2·06	·04	·02	39	28—39	..
			91·56	2·42	·46	2·52	·07	·04	52·5	..	..
Punnaku keerai		Mean	80·60	6·12	·67	2·51	·25	·038	35·65	48	145·9†
		Range	80·31	5·83	·36	2·30	·25	·03	32·58	39—57	..
			80·88	6·44	·67	2·46	..	·04	38·72	..	..
Kuppa keerai	*Amaranthus viridis*	Mean	81·75	5·17	·33	2·84	·32	·052	18·74	38	178·0†
		Range	81·30	5·07	·31	2·40	·32	·05	16·39	36·5	..
			82·21	5·30	·35	3·10	·34	..	20·98	40·0	..

* Ether extractive includes chlorophyll also.

† Vitamin C estimation done later on different sample.

II. *Studies of effects on young albino rats of single and combined supplements to the poor rice diet.*—

Studies of vitamin and calcium supplements: An experiment reported in 1946 (I.R.F.A. Report) showed (1) that the addition of 40 micrograms of riboflavin daily to the poor rice diet did not stimulate growth in young rats more than the rice diet alone, and that the riboflavin rats showed exaggerated deformities of spine and ribs (curvature, kyphosis, and collapse of ribs along the line of junction of dorsal and ventral elements) and (2) that the *combination* of adexolin and riboflavin caused excellent growth and normal skeletal development. Further experiments have confirmed these results with adexolin (100 I.U. of vitamin A and 16 I.U. of vitamin D per day) and riboflavin (40 micrograms per day) showing that *in combination* there was significantly better growth than would be predicted from the summed separate effects of adexolin and riboflavin. Further experiments, also of factorial design, with vitamins A and D instead of adexolin, singly and in combination with riboflavin and calcium lactate, have shown no consistent stimulating effect of A on growth, a marked effect of D, and in the only experiment so far concluded in which riboflavin and vitamin D were combined, a negative interaction between riboflavin and D of the 5 per cent level of significance. This is being tested further.

The effects of riboflavin alone as a supplement have been varied. It has been impossible with food shortage and rationing to secure sufficient stocks of rice to carry through comparable experiments on the same basal rice, and varying degrees of milling have affected the basal diet and the effect of supplementary riboflavin. It has sometimes depressed growth, sometimes increased it and sometimes had no effect. More deaths occurred during the experimental period among rats receiving riboflavin than among those on the unsupplemented rice diet (11 out of 21 with riboflavin as compared with 6 out of 18 on rice diet alone). Particular interest has centered in a possible relation, as suggested in the first experiment reported above on skeletal abnormalities, between riboflavin and calcium metabolism. Analyses of calcium and phosphorus in the humeri showed a marked diminution of calcium in the bone when riboflavin without additional calcium was added to the poor rice diet, and also a diminution, though less marked, in the percentage of phosphorus (Table II). This is being tested in further experiments and histological studies of bone are being made.

TABLE II.

Analysis of calcium and phosphorus in humeri of rats on poor rice diet with supplements of riboflavin and calcium lactate.

Group	Supplement	Litter, No. and sex.	Wt. at death, g*.	Length of humerus, g*.	Wt. of fat-free humerus, g.	Bone ash g./100 g.	Ca g./ 100 g.	P g./ 100 g.	Ca:P ratio
1	No supplement	A1 F	43·5*	1·65	·0369	42·83	10·50	9·15	1 : ·8714
		B9 M	62·0	1·69	·0186	46·77	10·21	8·06	1 : ·7894
2	Riboflavin 20 μg./ day	B5 F	71·0	1·88	·0431	30·86	6·19	5·49	1 : ·8869
		A7 M	71·0	1·88	·0435	33·11	8·51†	8·93	1 : 1·050
3	Riboflavin 40 μg. /day	B3 F*	44·5*	1·45	·0348	41·47	6·18	7·94	1 : 1·284
		A9 M	73·0	1·95	·0630	25·40	4·44	6·97	1 : 1·570
4	Calcium lactate 0·115 g. /day	C2 F	76·5*	1·66	·0536	51·86	11·57	10·73	1 : ·9274
		B7 M	86·0	2·12	·0689	46·88	11·46	11·52	1 : 1·006
5	Calcium lactate 0·23 g. /day	B1 F	106·0	2·20	·0830	56·62	10·93	13·01	1 : 1·191
		A8 M	88·0	2·20	·0770	58·05	9·02	10·74	1 : 1·191
6	Riboflavin 20 μg./ day *plus* cal. lactate 0·115 g. /day	C1 F	95·0	1·84	·0712	50·42	11·58	11·04	1 : ·9534
		C5 M	98·0	2·25	·0735	50·34	10·34	11·03	1 : 1·066
7	Ribo. 20 μg. /day *plus* cal.lactate 0·23 g. /day	A4 F	104·0*	1·86	·0717	59·41	21·96	10·25	1 : ·4668
		B6 M	135·0	2·18	·0806	55·55	10·79	11·37	1 : 1·046
8	Ribo. 40 μg./ day *plus* cal. lactate 0·115 g. / day.	A3 F	84·5*	1·81	·0627	51·36	12·75	11·04	1 : ·8658
		B2 M	112·0	2·22	·0897	55·97	11·31	10·16	1 : ·8982
9	Ribo. 40 μg. /day *plus* calcium lactate 0·23 g. /day	A2 F	101·0	1·99	·0879	56·54	13·66	10·72	1 : ·7852
		C6 M	96·0	2·18	·0777	56·37	15·44	11·72	1 : ·7593

* Rat B3 died after 7 weeks of experiment, age 77 days, and Nos. A1, C2, A4 and A3 were killed at same time. All others were killed at 9 weeks.

† Value based on only one sample.

8. Enquiry on the evaluation of proteins in terms of hæmopoietic activity and the relation of dietary protein to blood and muscle hæmoglobin under Professor M. Damodaran at the University Biochemical Laboratory, Triplicane, Madras.

The results of all previous experiments carried out under the scheme on the relation between dietary protein and the rate of hæmoglobin and r.b.c. regeneration in experimental anaemia have been collated and analysed and are presented in the accompanying Table.

The results prove conclusively that quality and quantity of protein exercises a decisive influence in blood regeneration and that in conditions of physiological and pathological stress feeding animal proteins at a high level is a necessary condition for efficient hæmopoiesis.

Experiments have been commenced for studying the effect of prolonged feeding with low protein diets on the blood picture and the blood and muscle hæmoglobin content. As these are long-term experiments they have not reached a stage at which conclusions can be drawn from them.

In the series of experiments that have been conducted in this Laboratory on the hæmopoietic activity of amino-acids experiments have been done on the role of histidine in blood regeneration in phenylhydrazine anæmia. From the experiments so far carried out histidine appears to be essential for normal blood regeneration.

TABLE

Increase in r.b.c. and hæmoglobin in 8 days at different level of casein, egg albumin, meat protein and wheat gluten.

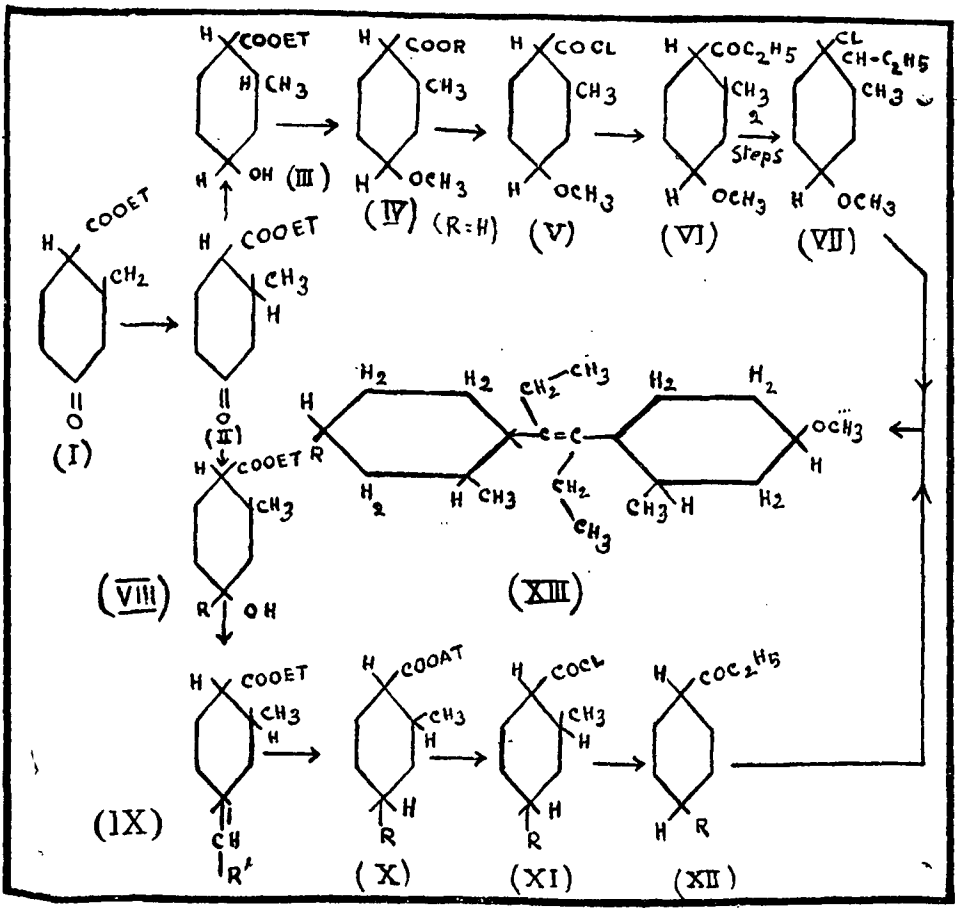
Expt. No.	Protein level, per cent.	No. of animals	R.b.c. in millions per c.mm.				Hb. in g. per 100 ml.				No. of days taken to come to normal
			4th day.	12th day	Increase	Percentage increase	4th day	12 th day	Increase	Percentage increase	
1.	Protein free	6	3·31	4·09	0·78	23·6±2·2	8·19	10·81	2·62	32·0±1·4	25
	Casein :										
2.	5	6	3·40	5·51	2·11	62·1±4·8	8·80	13·72	5·12	59·5±4·4	12
3.	15	6	3·62	6·66	3·04	86·1±3·9	8·31	14·48	6·17	74·7±2·6	10
4.	20	6	3·61	7·20	3·59	100·0±5·9	8·60	15·11	6·51	75·6±3·1	8
5.	25	6	3·63	7·37	3·74	101·2±4·2	8·23	15·07	6·84	77·9±1·8	8
6.	30	6	3·75	7·66	3·91	104·0±5·7	7·94	15·14	7·20	86·7±2·4	4
7.	35	6	3·77	7·69	3·92	104·0±5·6	7·75	15·28	7·53	97·1±4·6	4
	Egg albumin :										
8.	5	6	3·35	5·77	2·42	67·0±1·7	9·04	14·29	5·62	58·1±2·1	11
9.	15	6	3·51	6·92	3·41	95·7±3·7	7·82	14·37	6·55	84·6±3·2	10
10.	20	6	3·75	7·43	3·68	99·6±1·4	8·61	15·40	6·79	88·5±2·2	8
	Meat protein :										
11.	5	6	3·36	5·99	2·63	78·3±4·1	8·43	14·47	6·04	71·6±2·5	12
12.	15	6	3·71	6·90	3·19	86·0±2·9	8·50	15·17	6·67	78·5±1·6	8
13.	20	6	3·80	7·36	3·56	93·7±3·2	8·40	15·38	6·98	83·1±1·9	8
	Wheat gluten :										
14.	5	5	3·48	4·75	1·27	36·5±3·4	8·98	12·41	3·43	38·2±1·1	20
15.	15	6	3·58	5·34	1·76	49·2±3·6	8·98	13·67	4·69	46·9±2·9	11
16.	20	6	3·53	5·97	2·44	69·1±4·1	8·52	14·19	5·67	67·5±3·9	11
17.	Liver protein 5	6	3·59	5·54	1·95	54·3±4·8	8·54	13·22	4·68	54·7±5·1	14
18.	Rice protein 5	6	3·66	5·05	1·39	38·0±3·6	8·84	12·27	3·43	38·3±2·8	20

9. Enquiry on the preparation of anti-rachitic substances under Professor B. V. Bhide at the Maharajah Pratap Sinh Chemical Laboratory, S. P. College, Poona.

Part I.

In last year's Report the preparation of 1 keto tetrahydro $\triangle^{7:8}$ indene was described by making use of the Stobbe condensation of cyclohexanone with diethyl succinate. The yields of the product were, however, poor. The preparation of cyclohexadione by selenium dioxide oxidation of cyclohexanone also similarly proved to be difficult and toxic to the worker. Hence this line of attack was abandoned.

With a view to prepare a hydro-aromatic substance of the type (XIII) analogous to stilbo-estrol the following scheme is proposed :—



Ethyl-4-keto-2-methyl $\triangle^{2:3}$ cyclohexane carboxylate (I) is hydrogenated to give ethyl-4-keto-2-methyl-cyclohexane-carboxylate (II). (II) was reduced by aluminum isopropoxide and attempts were made to methylate the resulting hydroxy compound (m.p. 124°C. to 125°C.) with various reagents. The methylation proved to be unusually difficult. Methylation with diazo methane, methyl

iodide in acetone and potassium carbonate and dimethyl sulphate, potassium carbonate and acetone was unsuccessful. Ultimately, it was found that dimethyl sulphate and sodium hydroxide under reflux for a long time could effect methylation and hydrolysis though in a low yield to give 4-methoxy-2-methyl-cyclohexane carboxylic acid 140-150/6 mm. (IV R-H). Although the hydroxy acid is a solid the corresponding methoxy compound is a liquid. Further work according to the above scheme is in progress.

Ethyl-4-keto-2-methyl-cyclohexane 4-carboxylate (II) was treated with the grignard from isoamyl bromide, when the tertiary hydroxy compound (VIII) was isolated. Attempts made to de-hydrate (VIII) with potassium hydrogen sulphate, thionyl chloride and pyridine and iodine proved to be unsuccessful. Phosphorus pentoxide was, however, found to be suitable although each time only partial de-hydration occurred. On repeated treatment, however, the completely de-hydrated product (IX) could be isolated. This was hydrogenated to ethyl 4-amyl 2-methyl cyclohexane carboxylate (X). Further work is in progress.

PART II.

In continuation of the last year's work conditions for the preparation of 2-cinnamylidene-cyclohexanone and 2-citralydine cyclohexanone have been well established. Both these compounds have been subjected to Reformatsky's reaction with ethyl bromoacetate. The resulting hydroxy esters in each case could not be purified. They were hydrolysed and the crude hydroxy acids on boiling with acetic anhydride gave 1-methylene-2-cinnamylidene cyclohexane and 1 methylene-2-citralidene cyclohexane de-hydration and de-carboxylation having taken place simultaneously.

On similar lines, 4-acetoxy-cyclohexanone has been condensed with cinnamic aldehyde and on the experience gained in the previous work, proper conditions for the isolation of 4-acetoxy-2-cinnamylidene cyclohexanone (m.p. 170° C.) have been worked out. It has been characterized by its semicarbazone and oxime. Corresponding hydroxy compound has been prepared in a pure condition. It was observed that dicinnamylidene derivative was also simultaneously forming and decreasing the yield of the mono product. Because of the low yields the substance is being collected in quantity with a view to proceed further on lines already indicated. Further work is in progress.

Biological assay.— 1-methylene-2-cinnamylidene-cyclohexane and 1-methylene 2-citralidine-cyclohexane have been sent to Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor for biological assay and the results are awaited. The corresponding hydroxy compound will be sent for biological assay very shortly.

10. Enquiry on the nutritive value of soya-bean milk under Dr. S. S. De at the Indian Institute of Science, Bangalore.

Nutritive value of the protein.— In continuation of the earlier work, further experiments on the study of the nutritive value of soya-bean milk were carried out during the year. Earlier work carried out on rats had shown that for purposes of growth and for maintenance of nitrogen equilibrium, the protein of soya-bean milk was about 90% as efficient as cow's milk protein when the

proteins were fed at a 10% level. Further work on the nutritive value of the protein was carried out to study the comparative efficiency of soya-bean and cow's milk proteins for the following essential body processes :—

(i) Hæmopoesis after producing artificial anæmia according to Damodaran's method.

(ii) Maintenance of serum protein concentration of the blood.

(iii) Regeneration of the proteins of the liver after fasting for 48 hours.

The above methods have recently been used to assess quantitatively the nutritive value of a protein.

The results of the experiments are given in the Table :—

TABLE.

Comparative value of soya-milk protein and casein for hæmopoesis.

	Percentage increase over anæmic level	
	Hæmoglobin	R.b.c.
Casein ..	36·9	46·0
Soya-milk protein ..	33·0	43·9

Average composition of the serum of rats fed soya-milk protein and casein.

Protein source	Total nitrogen per 100 c.c. serum in mg.	Protein nitrogen in mg.	Albumin nitrogen per 100 c.c. in mg.	Globulin nitrogen per 100 c.c. in mg.	Albumin / Globulin
Casein ..	964·0	913·1	649·9	263·2	2·47
Soya-milk protein ..	937·0	886·1	622·8	263·2	2·39

Regeneration of liver proteins in rats fed soya-milk protein and casein.

(Protein expressed in terms of mg. of nitrogen per 100 g. of body-weight).

	Normal level.	Fasted level.	Regeneration after re-feeding for	
			2 days	4 days
Casein ..	111·9	83·3	108·9	110·4
Soya-milk protein	111·9	83·3	105·9	105·6

The data presented in the Table show that soya-milk protein is about 85 to 90% as good as casein if its nutritive value is determined by the above methods. These results also corroborate the previous work which had shown that soya-bean milk is about 90% as good as cow's milk regarding the nutritive value of the protein.

The work so far done on the nutritive value of soya-bean milk had been carried out on rats only. The next series of experiments was carried out to confirm the above results by experiments on human subjects. In the first

experiment the actual digestibility coefficient and biological value of soya-bean milk protein compared with egg protein were determined on two human subjects according to the method of Mitchell. Compared with egg protein, the digestibility coefficient and the biological value of soya-milk protein were 96·6 and 94·0, respectively.

A second series of experiments on six human subjects were carried out to study the economy of utilization of the proteins of soya-bean and cow's milk curds when fed along with a poor South Indian rice diet. The curd proteins were fed for one week periods on the subjects whose basal diet consisted chiefly of rice protein. The curd proteins formed about 1/7th of the total protein intake. The data obtained by the experiment showed that the protein of soya-bean curd was about 80% as good as cow's curd proteins when fed along with the basal diet.

The above results on the nutritive value of the protein of soya-bean milk show that it compares favourably with a high-class animal protein like that present in milk or egg.

Utilization of calcium by growing children.— Comparative studies in the utilization of calcium from fortified soya milk and cow's milk were carried out with six children (aged 7 to 9 years) who received the milks as supplement to a basal diet, composed mainly of cereals and which provided only a small amount of calcium (224 mg.) per day. The children showed a negative calcium balance with the basal diet, on supplementing the diet with fortified soya on cow's milk, the children received about 365 mg. of calcium per day. Due to this supplementation the calcium balance was changed from an average of—54 mg. per day in the case of basal diet to about +29 mg. in the case of both soya milk and cow's milk. The percentage utilization of the supplementary calcium was 23·1% in the case of soya milk and 22·8% in the cow's milk.

Hœmatopoietic activity of iron in soya milk and its utilization.—The hæmoglobin regeneration of rats rendered anæmic by prolonged feeding of milk diet was determined by feeding soya milk with ferric chloride as control. Soya milk contain ·13 mg. of iron per litre. The hæmoglobin response with soya milk was 89 per cent of that with equivalent amount of ferric chloride. The percentage of iron utilized was 49·5 per cent with soya milk compared to 54 per cent in the case of ferric chloride.

Maltavena.—The formula for the production of malted infant food suitable for use in supplementing milk in countries where an acute shortage may occur was originally devised Dr. Caprino working in the laboratories of Paroni's breweries. The original Caprino formula made use of barley malt and oat flour in certain proportions, modified Caprino's formula use barley malt, wheat flour with soya flour or skim-milk powder. The feeding trials with maltavena in hospitals, orphanages and other institutions indicate that this can be safely fed to infants aged above three months and can be maintained safely on this for a few months. Supplemented with little milk the food value is enhanced to a great extent. The preparation of maltavena from indigenous sources was taken up. Following roughly the Caprino formula preliminary experiments were carried out with different malts like barley, ragi and jowar. In the place of oat flour and wheat flour, Bengal-gram flour and soya flour was used. Finally, a preparation from ragi malt (70%), Bengal-gram flour (20%), and skim-

milk powder (10%) was evolved having a good taste and highly nutritive. The analysis of maltavena prepared with above composition was as follows:—

Total solids	..	15·9%	Calcium	..	0·1002 microgram.
Protein	..	1·93%	Thiamine	..	19·9 ,,
Ash	..	0·44	Riboflavin	..	28·8 ,,
Reducing sugar	..	9·3	Nicotinic acid		1,100 ,,
Dextrins	..	2·48	Fat	..	*Nil.*

The nutritive value of the protein and B-complex vitamin contained in maltavena was compared with that of skim-milk powder by growth method on young rats. Both the diets were supplemented with fat (4% on solid basis), minerals and vitamins A and D. The skim-milk powder was so diluted that it contained the same amount of protein as in maltavena. Sucrose was added to skim-milk diet to make up the solid content. Group of six rats were fed on this diet for a period of ten weeks and the results obtained was as follows:—

Diet		Protein content	Average weekly increase in weight (in g.)
Maltavena	..	1·93	7·45
Skim milk	..	1·93	7·30

The above results indicate that as a source of protein and B-complex vitamin maltavena is slightly superior to that of skim milk having some protein concentration.

Nutritive value of soya curd.—The biological value of the protein of soya curd as determined by metabolism method was found to be 76 and that of the milk curd 82. The supplementary value of the curds as supplement to poor rice diet was as follows:—

		Weekly increase in weight (in g.)
(i) Poor rice diet	..	3·5
(ii) ,, ,,	+10 c.c. of soya curd ..	6·8
(iii) ,, ,,	+10 c.c. of milk curd ..	7·2

The development of acidity on souring with various sugars to soya milk was also studied. Addition of lactose or invert sugar produced acidity of the same order as produced on souring cow's milk under the same conditions. The acidity develops a maximum at about 48 hours and then declines. The viability of different organisms such as typhoid, dysentery and cholera in soya curd has been studied. The results were found to be similar to that of cow's milk curd as has been found by Panja and Ghosh (*I.M.G.*, 1945, **80**, p. 300).

Nutritive value of the residue.— The residue after preparation of milk contain more than 55 per cent protein. The residue on drying in hot air and powdering gives a good flour having no smell of the original bean. Nutritive value of mixtures of wheat flour and different proportions of this flour were studied and the following results were obtained:—

	Gain in weight per g. protein consumed. Grammes.
Wheat flour alone ..	0·85
,, ,, +(90)+residue (10) ..	1·85
,, ,, +(80)+residue (20) ..	2·1

Bread, biscuits and other preparation made with wheat flour and residue in the proportion of 80 : 20 were quite tasty and very pleasant in appearance. The residue which is about 25% of the original bean can be used for admixture with wheat and gram flours, thereby increasing their nutritive value.

Flavour.—Soya milk even produced by improved method has a nutty flavour. Attempts were made in various directions to remove the last trace of the beany flavour. Steaming for a short time improves the flavour to a certain extent. Addition of dilute solution of coumarin or vanillin also mask the beany flavour. Steeping with dilution of sodium sulphate overnight and then extraction with sodium bicarbonate also provides better results. The best results have been, however, obtained on incubation with certain types of curd and cheese cultures and a flavour resembling that of milk flavour has been produced in the soya milk.

Feeding experiment on infants and toddlers.—The experiment in Municipal Creches, C. & M. Station, Bangalore which was started in May 1946 has completed one year. The experiment was started with 120 subjects, but 15 left the Creche before the end of the experiment.

The number of subjects up to end of the experiments were as follows :—

Infants on cow's milk	..	11
Infants on soya-bean milk	..	8
Toddlers on cow's milk	..	40
Toddlers on soya-bean milk	..	45

The weights and heights were recorded every month, but that recorded in September 1946, November 1946 and April 1947 were considered for analysing the results. The period from May 1946 to September 1947 is indicated as first period, from May 1946 to November 1946 as second period, and similarly the third period. The results for the toddlers are statistically analysed as follows :—

	1st period.	2nd period.	3rd period.
Cow's milk ..	2·28 ± 0·21	2·70 ± 0·27	400 ± 0·33
Soya-bean milk ..	1·58 ± 0·21	2·28 ± 0·27	3·51 ± 0·30
Difference ..	0·59 ± 0·29	0·42 ± 0·38	0·49 ± 0·45

Except in the first period the differences are not significant as can be seen from the Table that magnitude of the error increases with the absolute value of the means and the differences diminish with the progress of the experiment. Though the effect of the soya-bean milk is far behind that of cow's milk in the first period, in later period it is able to make up as can be seen from the values of the differences to the absolute value of the mean increase due to cow's milk.

The difference between the two milks in percentage of mean increase due to cow's milk is as below :—

1st period.	2nd period.	3rd period.
30·3	15·6	12·3

Infants.—With the number of subjects under this group no statistical analysis can be made but the following Table is presented to show the mean increase in weights of infants over the different periods :—

	1st period.	2nd period.	3rd period.
Cow's milk ..	2·60 (11)	3·02 (11)	2·86 (9)
Soya-bean milk ..	2·84 (8)	3·72 (8)	4·70 (8)

Human feeding experiment with soya-bean milk.—The main human feeding experiment is being conducted at present with 400 children in local orphanages. The preliminary experiment was started in February 1947 with 120 children with a view to get an idea on the rate of growth and the dosage of milk that will be required to produce statistically significant results. The main experiment was started in June in consultation with Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor, and Prof. C. Chandra Sekar, Statistician, All-India Institute of Hygiene & Public Health, Calcutta. The present plan of the experiment includes four groups:—

Group A (95 children)	.. ..	12 oz. of cow's milk.
Group B (154 children)	.. ..	12 oz. of soya milk.
Group C (95 children)	.. ..	9 oz. of cow's milk.
Group D (56 children)	.. ..	Control.

In addition to recording of weights and heights systematic record is maintained of nutrition deficiency in a score card and also r.b.c. and hæmoglobin level at periodical intervals. From the results of the experiment, it indicates that soya milk is more nutritive than 75 per cent dose of cow's milk.

11. Enquiry on plant carotenoids in relation to their biological importance under Dr. Bashir Ahmad at the Laboratories of the Council of Scientific & Industrial Research, University Buildings, Delhi.

This enquiry which has been conducted for the last seven months only, is an extension of the investigations carried out on the vitamin A activity of plant foods. The determination of the vitamin A activity of most of the common plant products was completed last March, by isolating and estimating the amounts of individual carotenoid pigments present in each material. That investigation raised a very important and relevant problem, namely, the carotenoid pigments actually present in the plant cells and those estimated by the isolation since the pigments isomerize quite rapidly and significantly when taken out of their natural environment. Further, what are different isomers which result from the active pigment and what exactly is their biological activity as compared to that of B-carotene. Further still what natural changes occur in the carotenoid pigments during the normal course of the development of plants. These problems are not merely of academic interest but an insight into these is likely to be of real practical value for their synthetic production and the conversion of some of the inactive ones into those which are physiologically active.

While investigating the first question a large number of experiments was conducted from which it appears to be fairly certain that the neo-isomers do not occur in the plant cells but are formed during the process of isolation. Samples of neo-B-carotene-B and neo-B carotene-U were then prepared with all possible care and in a fair degree of purity. The biological value of these isomers has been subject of 4 different investigation by experiments on albino rats.

On account of the extreme hot weather of Delhi even the control animals on B-carotene did not show normal growth, but in all the four experiments the average growth of the animals on B-carotene B was half of the average growth shown by the animals on pure B-carotene, and the average growth in the two groups was comparable when the dose of the neo-B-carotene B was twice that of pure B-carotene. The results of experiments with neo-B carotene-U were

inconclusive. All these experiments are being repeated in the cold weather when it is hoped that more satisfactory results will be obtained.

The changes that occur in the carotenoid pigments during different stages of development have been studied in the different leafy materials in the tomato and the mango. A mass of data has been obtained which is being studied to determine significant trends. These will be presented later. The work is also being further continued in the next spring and the summer it will be extended to a number of varieties of mango and it is hoped that something of interest will emerge.

During the last six months another problem of considerable practical importance has been studied. This was the stability of B-carotene in vanaspati during storage under different conditions. Since it is being recommended that vanaspati should be vitaminized for vitamin A, it is of considerable importance to know whether B-carotene will be stable in this medium to a reasonable degree with or without the addition of anti-oxidants.

The same sample of carotenized vanaspati was stored under different conditions, namely, in the light, in dark, in tin, in glass bottles, in cold, at a room temperature, without anti-oxidants and with the addition of ethyl gallate or α tocopherol as anti-oxidant and so on. Determination of the different isomers of carotenoids was made at the intervals of 1 week, 2 weeks, 4 weeks, 8 weeks and 12 weeks. Curiously enough carotene was fairly stable under all conditions even with or without anti-oxidants. It is possible that carotene concentrates which was added already contained anti-oxidants. This experiment will be repeated under more controlled conditions and with known methods of production of both vanaspati and the carotene concentrates.

12. Enquiry on the growth-promoting factor present in Bengal gram under Dr. Bashir Ahmad at the Laboratories of the Council of Scientific & Industrial Research, University Buildings, Delhi.

This enquiry started in April this year and work has been conducted for the last six months only. So far three experiments have been conducted on a number of groups of young albino rats as experimental animals which received a basal diet containing 4% torula yeast, cod-liver oil, hydrogenated vegetable fat and a salt mixture in addition to the common ingredients. The diet was presumably adequate in all the known nutritional factors.

In the *first experiment* there were five groups of six animals each receiving the supplements shown below in addition to the basal diet for a period of 8 weeks during which their rate of growth and general well-being were observed :—

(i) Control group on basal diet alone.

(ii) A supplement of 3 g. gram (powdered ungerminated gram).

(iii) A supplement of 3 g. germinated Bengal gram.

(iv) 2·8 g. germinated gram the germ having been removed.

(v) 0·2 g. germ excise out of 3 g. of germinated gram.

In this experiment the growth of the animals was generally poor the average being only 1·4 g. per week in the control animals. It increased to 4·45 g. with the addition of gram; to 4·0%g. with germinated gram without germ ;

to 7·2 g. with whole germinated gram; and to 8·1 g. with the germ alone. The effect of the germinated gram particularly the germ was very marked but the poor growth of the control animals pointed to inadequacy of thiamin which was made good by the germinated gram and the germ.

In the *second experiment* three groups were fed the following supplements on the same basal diet :—

(i) 0·3 g. of germ excised out of germinated gram.

(ii) 20 μg. of an alcoholic extract of the germ from germinated gram.

(iii) 150 μg. of biochanin C (Siddiqui *et al., Jour. Sci. Ind. Res.*, **4**, p. 68).

The average growth rates in the three groups were 10·6 g., 9·5 g. and 10·3 g. per week, respectively. This experiment, while partly supported the conclusions of the first, showed curiously enough that even biochanin C which had no relation to thiamin or any other known nutritional factor, produced the same growth-stimulating effect as the germ or the alcoholic extract of germ, in which the effect could be ascribed to thiamin or other B-group vitamins.

In the *third experiment* 8 groups of animals were used with the same basal diet but supplemented in some cases with additional 4% of food yeast or medicinal yeast. The various groups with the average rate of growth per week of animals are shown below :—

		Average rate of growth in grammes.
(i)	Basal diet+4% food yeast	10·0
(ii)	Basal diet+4% food yeast+0·2 g. germ ..	13·0
(iii)	Basal diet+4% food yeast+150 μg biochanin C	13·2
(iv)	Basal diet+4% food yeast+3 μg thiamin ..	14·0
(v)	Basal diet+4% food yeast+3 μg thiamin +0·2 g. germ ..	13·0
(vi)	Basal diet+4% food yeast+3 μg thiamin +150μg biochanin C ..	14·0
(vii)	Basal diet+4% medicinal yeast ..	8·0
(viii)	Basal diet+4% medicinal yeast+3 μg thiamin +150 μg. biochanin C ..	14·0

The addition of further amounts of food yeast and medicinal yeast increased the growth rate. To this food mixture when pure thiamin or germ were added almost the maximum growth rate was obtained. At the same time, the same effect was obtained when biochanin C was fed without adding any thiamin or germ. The addition of biochanin C when the animals were already receiving pure thiamin had no further growth-stimulating effect.

Biochanin C which is not indentical with any known vitamin seems to act as a growth-stimulating factor. The issues are known still involved and further work on this interesting problem is being continued.

13. Bangalore School Feeding Scheme under Prof. V. Subrahmanyan at the Indian Institute of Science, Bangalore.

This experiment had to be planned side by side with the feeding experiments in orphanages where the children receive as much as 12 ounces of milk per day on all days of the week. It was felt that the latter series would yield

results of more scientific and practical value, and so in concurrence with the Director of the Nutrition Research Laboratories, Coonoor and the Professor of Statistics of the All-India Institute of Hygiene & Public Health, Calcutta the medical investigator deputed from Coonoor, was asked to concentrate primarily on the experiments with soya milk and cow's milk in the orphanages. It is also decided that the observations on the school children receiving curd and rice could be started at a later date after organizing the work in the orphanages.

Accordingly, the systematic observation in connection with the present scheme was commenced on 1st July, 1947 with the children in one big institution over thousand students. Children ranging in age from 8 to 15 were selected and their midday meals were arranged as follows :—

(i) One hundred and twenty-five children to receive six ounces of separated-milk curd and seven ounces of cooked rice.

(ii) One hundred and twenty-five children to receive six ounces of soya-milk curd and seven ounces of cooked rice.

(iii) One hundred and twenty-five children of the same age group, who were going home for their midday meals were maintained as controls.

In the initial stages before the systematic observations were commenced, the children in each group were receiving only eleven ounces of the curd and rice mixture per child consisting of four ounces of curd and seven ounces of cooked rice. It being found that the quantity was rather insufficient and in order to obtain a more favourable response of children getting the curd and rice mixture, it was decided to increase the total amount from eleven ounces to thirteen ounces by providing an extra two ounces of separated-milk curd or soya curd for each child per day.

Shortly after the experiment was commenced, the political and other troubles in Mysore State started and school-going children were the first to be affected. The schools were compulsorily closed up to the end of October 1947, so much so that there was a long break in the feeding arrangements. The schools were recently re-opened, but as they will be closing again in about a fortnight it was considered desirable to make a fresh start only in the month of January 1948. According to the present programme, the feeding experiment can be continued only up to 31st March, 1948.

The preliminary observations made by the Health Officer of the Civil Station and his staff have already been communicated to the Food Department. Observations over a period of even two months had shown that the children receiving either soya-milk curd and rice or separated-milk curd and rice are generally healthier and put on more weight than those who proceeded to their homes for their midday meals. Our observations are unfortunately, still incomplete.

School-feeding experiments have the defect that the feeds can be given only on working days and that we have no control over what the children eat in their homes. There are also liable to be sudden closures, changes from one school to another and such unforseen circumstances which affect the practical value of any observation to be made. In spite of all these, midday feeding has come to be recognized as an elegant method of providing essential supplements to the vulnerable groups. Systematic observations on children receiving the benefit of such feeding would be very useful.

List of papers published.

DATTA, N. C., and CHAKRAVARTY, R. K.—Plasma Proteins in Health and Disease. Part I. Plasma Proteins in Healthy Individuals (*I. J. M. R.*, **35**, No. 4).

DATTA, N. C.—Plasma Proteins in Health and Disease. Part II. Fractionation of Plasma Proteins in Hyperprotenæma (*I. J. M. R.*, **35**, No. 4).

DESIKACHAR, H. S. R., DE. S. S., and SUBRAHMANYAN, V.—Studies on the Nutritive Value of Soya-bean Milk—Part I (*Annals Biochem. & Exper. Med.*, **6**, No. 2, p. 49).

DESIKACHAR, H. S. R., DE, S. S., and SUBRAHMANYAN, V.—Studies on the Nutritive Value of Soya-bean Milk—Part II (*Annals Biochem. & Exper. Med.*, **6**, No. 2, p. 57).

DESIKACHAR, H. S. R., DE, S. S., and SUBRAHMANYAN, V.—Studies on the Nutritive Value of Soya-bean Milk—Part III (*Annals Biochem. & Exper. Med.*, **6**, No. 2, p. 62).

DE, S.S., DESIKACHAR, H.S.R., KARNAI, B.T. and SUBRAHMANYAN, V. —Investigations on Soya-bean Milk and Related Products, (*Sci. & Culture*, **12**, p. 587).

GOPALAN, C.—The Ætiology of Phrynoderma—(*Ind. Med. Gaz.*, **82**, No. 1, Jany., 1947).

DIKSHIT, P. K., and PATWARDHAN, V. N.—Studies in Experimental Rickets. The Alkaline Serum. Phosphatase in Rachitic Albino Rats (*I. J. M. R.*, **35**, 2, April 1947, p. 91).

ROY S. C., ROY, S. K., and GUHA, B. C.—Role of Thiamin and Riboflavin in the Biosynthesis of Vitamin C (*Nature*, 1946, **158**, p. 238).

ROY, S. C.—Studies in certain Aspects of the Biosynthesis of Vitamin C.

SADANA, J. C., and BASHIR AHMAD.—The Carotenoids Pigments and the Vitamin A Activity of Indian Carrots (*I. J. M. R.*, **35**, No. 2).

SAHASRABUDHE, M. R., DESIKACHAR, H. S. R., and LAKSHMI-NARAYANA RAO, M. R.—Biological Value of Soya-bean Milk Protein by Regeneration and Liver Protein (*Curr. Sci.*, October 1947).

SUNDARARAJAN, A. R.—Vitamin A in Fish-liver Oils and Carotene in Food-stuffs (*Curr. Sci.*, **16**, No. 3, March 1947).

Some Rich Sources Active Carotenoids, (*Jour. Sci. Ind. Res.*, July 1947).

Studies in Nutritive Value of Cereals and Pulses (*in press*).

Studies in Experimental Rickets (*I. J. M. R.*, **34**, p. 263).

Studies in Experimental Rickets (*I. J. M. R.*, **35**, p. 81).

REPORT OF THE SIXTEENTH MEETING OF THE NUTRITION ADVISORY COMMITTEE OF THE INDIAN RESEARCH FUND ASSOCIATION HELD IN NEW DELHI ON THE 8TH DECEMBER, 1947.

COMPOSITION OF THE COMMITTEE.

PRESENT.

Dr. G. Sankaran, Professor of Biochemisty and Nutrition, All-India Institue of Hygiene & Public Health, Calcutta. (*Chairman*).

Professor S. P. Agharkar, Head of the Department of Botany, Maharastra Association for the Cultivation of Science, Law College, Poona.

Dr. S. Banerji, Diabetes Research Worker, School of Tropical Medicine, Calcutta.

Dr. K. P. Basu, Dairy Chemist, Indian Dairy Research Institute, Hosur Road, Bangalore.

Dr. B. C. Guha, Professor of Applied Chemistry, Calcutta University, 92 Upper Circular Road, Calcutta.

Dr. C. O. Karunakaran, Superintendent, Public Health Laboratory, Trivandrum.

Dr. R. B. Lal, Professor of Epidemiology of Malaria, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. T. Bhaskara Menon, Principal, Andhra Medical College, Vizagapatam.

Dr. V. N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor. (*Secretary*).

Dr. Jivraj N. Mehta, Chairman of the Scientific Advisory Board, kindly attended the meeting.

Mr. B. N. Adarkar, Dr. Bashir Ahmad and Sir Shanti Swarup Bhatnagar were unable to attend.

The following were present by invitation :—

Major P. D. Bhave, Officer on Special Duty (Nutrition), Central Provinces & Berar, Nagpur.

Lieut.-Colonel Barkat Narain, Director of Health Services, Delhi Province, Delhi.

Miss M. Craig, Principal, College of Nursing, Delhi.

Major M. S. Chadha, Chief Health Officer, Delhi Province, Delhi.

Major-General A. C. Chatterji, Director of Health Services and Inspector-General of Prisons, West Bengal, Calcutta.

Dr. R. G. Dhayagude, Dean, Seth G. S. Medical College, Bombay.

Professor M. Damodaran, Director, University Biochemical Laboratory, Madras.

Dr. S. S. De, Lecturer in Food Technology, Indian Institute of Science, Bangalore.

Dr. R. E. Dadachanji, Nutrition Officer, Finance Department, Civil Supplies Department, Bombay.

Professor P. V. Gharpure, Professor of Pathology, Grant Medical College, Bombay.

Mr. G. Ford, Factory Manager, The Hindustan Vanaspati Manufacturing Company Limited, Sewri, Bombay.

Dr. C. R. Das Gupta, In-charge, Hæmatology Research Department, School of Tropical Medicine, Calcutta..

Mr. T. Gonsalves, Assistant Secretary, Indian Research Fund Association, New Delhi.

Dr. P. V. George, Nutrition Officer, Public Health Department, Madras.

Dr. S. W. Hardikar, "Abhas", Hardikar Bagh, Himayatnagar, Hyderabad (Deccan).

Dr. V. R. Khanolkar, Director of Laboratories, Tata Memorial Hospital, Bombay.

Dr. N. D. Kehar, Officer-in-Charge, Animal Nutrition Section, Indian Veterinary Research Institute, Izatnagar.

Dr. D. V. Karmarkar, Technical Adviser, Ministry of Food, Government of India, New Delhi.

Lieut.-Colonel C. K. Lakshmanan, Director, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. K. Mitra, Office of the Director-General of Health Services, New Delhi.

Dr. A. R. Mehta, Director of Public Health, East Punjab, Simla.

Lieut.-Colonel T. C. Puri, Deputy Director-General of Health Services, Government of India, New Delhi.

Dr. K. Rajagopal, Associate Professor of Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. D. W. Soman, Assistant Director, Haffkine Institute, Bombay.

Professor V. Subrahmanyan, Professor of Biochemistry, Indian Institute of Science, Bangalore.

Dr. K. C. Sen, Director, Indian Dairy Research Institute, Hosur Road, Bangalore.

Major O. P. Verma, Officer Commanding, Military Food Laboratories (India), New Delhi.

Dr. N. Veeraraghavan, Director, Pasteur Institute, Coonoor.

1. To Review Work in Progress and Consider the Question of Continuation or Otherwise of Existing Enquiries.

(A) The Committee recommended the continuation of the following enquiries :—

(i) Nutrition researches at the Nutrition Research Laboratories, Coonoor.

(ii) Ascorbic acid enquiry under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

(iii) Enquiry on the formation, function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay.

(iv) Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical, College, Bombay.

The Committee recommended that the work on vanaspati may be co-ordinated with similar work carried on elsewhere..

(v) Enquiry on the chemical nature and nutritional availability of food iron under Dr. B. C. Guha at the University College of Science & Technology, Calcutta.

(vi) Enquiry on poor rice diets and food preparations in common use in South India under Dr. (Miss) Eleanor D. Mason at the Women's Christian College, Madras.

(vii) Enquiry on the evaluation of proteins in terms of hæmopoietic activity and the relation of dietary protein to blood and muscle hæmoglobin under Professor M. Damodaran at the University Biochemical Laboratory, Triplicane, Madras.

(viii) Enquiry on the preparation of anti-rachitic susbtances under Professor B. V. Bhide at the Maharaja Pratap Sinh Chemical Laboratory. S. P. College, Poona.

(ix) Enquiry on the nutritive value of soya-bean milk under Dr. S. S. De at the Indian Institute of Science, Bangalore.

(x) Bangalore School Feeding Scheme.

The Committee recommended that a sum of Rs. 24,468 be provided for the above two schemes and that the work be completed during next year.

Nutrition research unit under Professor V. Subrahmanyan and Dr. S. S. De.

The Committee recommended that the staff engaged on the soya-bean enquiry may be absorbed in the nutrition research unit which may start in 1949-50 with a programme of research to be approved by the Committee.

(xi) Enquiry on plant carotenoids in relation to their biological importance to be continued for one year under the supervision of Dr. K. Rajagopal, Associate Professor of Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

(xii) Enquiry on the growth-promoting factor present in Bengal gram to be continued under the supervison of Dr. M. Damodaran, Director, Biochemical Laboratories, University of Madras, Madras.

(B) (i) The continuation of the nutrition research unit under Dr. H. N. De, at Dacca University, Dacca, was not recommended.

(ii) The Committee recommended that the Government of Madras may be requested to take over the nutrition research clinic at the Stanley Medical Hospital, Madras, after 1947-48.

2. To Consider New Proposals.

(A) The Committee recommended the following new enquiries :—

(i) Enquiry on the metabolism of vitamin A under Dr. K. Rajagopal, Associate Professor of Nutrition, All-India Institute of Hygiene & Public Health, Calcutta.

(ii) Enquiry on the role of fat in lactose metabolism under Dr. S. Banerjee, Diabetes Research Worker, School of Tropical Medicine, Calcutta.

(iii) Enquiry into the factors involved in the biosynthesis of fat with special reference to thiamin and riboflavin under Dr. B. C. Guha,

Head of the Department of Applied Chemistry, University College of Science & Technology, Calcutta.

(iv) Nutrition research unit under Dr. B. C. Guha, Head of the Department of Applied Chemistry, University College of Science & Technology, Calcutta.

The Committee recommended that all the existing and proposed enquiries under Dr. Guha should be absorbed into this unit and that Professor Guha may be requested to submit a revised budget of the order of Rs. 25,000 to the Scientific Advisory Board.

(B) The following enquiry was not recommended:—

Enquiry on the fortification of edible fat with vitamin A under Dr. U. P. Basu, Officer-in-Charge, Bengal Immunity Research Laboratory, Calcutta.

3. To Consider Letters from Dr. B. C. Guha, D.Sc., F.N.I., Head of the Department of Applied Chemistry, University College of Science & Technology, Calcutta, on the Recommendations made by the Nutrition Advisory Committee at its Meeting held on the 9th December, 1946 regarding the Enquiries entitled (a) Ascorbic Acid Enquiry and (b) Enquiry on the Chemical Nature and Nutritional Availability of Food Iron at the University College of Science & Technology, Calcutta.

The Committee considered these letters and decided to place on record that if the programme of work of any existing enquiry was not found sufficiently satisfactory, the Committee may suggest a new approach to the problem and make some constructive suggestions unless it wished to recommend the discontinuation of the enquiry. Mere generalized statements were not desirable.

4. To Approve the Publication of the Note on the Results of Diet Surveys in India, Burma and Ceylon (Para 3 of the Proceedings of the Meeting of the Nutrition Advisory Committee held on the 28th November, 1945).

The Committee noted that several diet surveys recently carried out in different parts of India had not been included in the note submitted to them. This left important lacunæ in the information proposed to be presented with the result that the conclusion reached might not be completely valid. The Committee, therefore, recommended (a) that the note submitted to the Committee may be published and (b) that a Sub-Committee consisting of Dr. K. Mitra (Convener), Dr. Dadachanji, Dr. Rajagopal, Dr. S. Banerjee and Dr. Karunakaran may be asked to bring the information up to date and present a more accurate picture in a fresh note the draft of which would be considered by the Nutrition Advisory Committee at its next meeting.

5. To Consider the Nutritive Value of Vanaspati.

The Committee considered the notes submitted by Dr. Bashir Ahmad, Dr. Basu, Dr. Guha and Dr. Patwardhan. The Committee noted with

pleasure that the Vanaspati Research Planning Committee of the Ministry of Food had undertaken extensive and co-ordinated investigations on the problem. The Committee considered it desirable, however, that the workers interested in the question should be invited to the meetings of that Committee. The Committee further felt that discussion on vanaspati should be postponed till the results of those investigations are available.

6. Co-ordination of Research.

The Committee recommends that in order to co-ordinate research on similar problems the workers interested should keep in close touch with each other and meet whenever it is considered necessary. Such meetings might preferably be arranged one or two days prior to the meetings of the Nutrition Advisory Committee.

7. Nutrition Studies on Refugees.

The Committee is of the opinion that it would be desirable to carry out studies on the nutritional status of the inmates of refugee camps, if conditions permit.

8. Evaluation of Nutritional Status.

The Committee is of the opinion that the adoption of uniform methods for the evaluation of nutritional status of population groups in India is desirable. Since the question is of great importance to the country, the Committee recommends that a Sub-Committee consisting of Dr. G. Sankaran (Convener), Dr. Bhave, Dr. Mitra, Dr. Karunakaran and Dr. Patwardhan with powers to co-opt be asked to study the problem and submit their report for consideration at the next meeting of the Nutrition Advisory Committee.

9. To Consider the Progress of Nutrition Work in Provinces.

Assam.

One diet survey in Palasbari town on 12 families with 93 people has been carried out. The Palasbari town is a business centre for local products. It has both the urban and the rural elements amongst its population. The results of the survey showed that the diets which provided about 2,400 calories followed a pattern similar to that observed in other rice-eating regions.

Madras.

The nutrition activity in the Province during the year 1947, ranged over the field of diet and nutrition surveys, ameliorative measures to improve the nutrition of vulnerable groups, nutrition education, training of personnel, etc.

Diet and nutrition surveys.—The diet surveys carried out during the year included 1,300 families and students' hostels in 83 different localities in the districts of Trichy, Chittoor, Kurnool, Anantapur, Chingleput, Ramnad, South Arcot, Tinnevelly, Salem, Madras, Malabar, North Arcot, Nellore and Cuddappah. In general the diets were found to be deficient in quality as well as in quantity. In certain districts the caloric intake was distinctly low and was almost on starvation level. The other features of the diets were similar to the poor rice eaters' diet already fairly well known. Under- and malnutrition is, therefore, largely prevalent in all the areas surveyed. Deficiencies of vitamins A and of B-complex were more common.

Ameliorative measures.—Free midday meals to nearly 67,000 children attending the labour schools were provided by the Government of Madras. The Madras Corporation continued midday meals to about 7,000 children attending the Corporation Schools. The Government scheme for free midday meals to elementary school children, however, was discontinued in the year under review. Free distribution of milk to infants, expectant and nursing mothers is being continued in some centres. The production of milk re-constituted from separated milk powder at the Government milk factory is relieving to a certain extent the acute milk shortage. The Government is also distributing cod-liver oil, calcium lactate and multi-vitamin tablets free of charge.

Nutrition education.— Education in nutrition is being imparted to the public by means of exhibitions, radio talks and publications of articles and pamphlets. The Central Bureau of Nutrition at Madras is being equipped to undertake the training of personnel for the purpose of extending the activities of the nutrition department.

Bihar.

Diet surveys.—A total of 196 Harijan families were investigated with regard to their dietary habits. The results of the survey showed that intake of all the protective foods was uniformally low in all the groups and at all the places. Over a thousand children from the same group were examined for their state of nutrition and a large percentage of these children were found to be mal-nourished although the prevalence of frank deficiency disease was low.

The Department is undertaking propaganda and education in nutrition through exhibitions and publications of pamphlets on the subject.

Central Provinces & Berar.

Lathyrism.—No fresh attacks of lathyrism were reported during the last six months or so. The Teora Control Order of 1946 had the desired effect in preventing the further attack of lathyrism. The Government is expected to sanction the joint scheme of investigation put up to it by the Indian Research Fund Association last year and the investigation is expected to commence in the financial year 1948-49.

School-feeding experiment.—A beginning with the experiment in school-feeding schemes has been made in certain selected schools. The foods chosen for supplementation were : (i) sprouted gram, (ii) ground-nut (iii) separated-milk powder, (iv) multi-vitamin tablets, (v) a combination of (iii) and (iv) and (vi) shark-liver oil. Sprouted gram and ground-nut showed little advantage over the controls. Separated-milk powder and multi-vitamin tablets gave better results. A combination of the two latter, however, gave still better results adjudged by the increase in heights and weights of the children under experiment.

The activity of the Nutrition Department regarding propaganda and education was kept up in the last year. Several exhibitions were held in the Province in food and nutrition lectures were delivered and pamphlets published.

Diet surveys on the agricultural population in certain districts and on aboriginees have also been carried out.

Bombay.

The food situation in the Province remained uncertain throughout the year. The Government explored all possible avenues of augmenting the food

supply particularly with regard to milk, fish, vegetables, etc. The Government distributed free of charge multi-vitamin tablets prepared by the Haffkine Institute, Bombay, as in the earlier years. Community feeding is being encouraged in large Government establishments and industrial concerns. There are 179 industrial canteens catering for meals, teas and snacks. These are proving very popular. There has not been much progress in the school-feeding programme, however, because of the continued food shortage and financial difficulties. A rapid clinical nutrition survey of 10,000 school children of the Primary Schools of the city of Bombay has been carried out during the year.

Nutrition education is being imparted to the public through exhibitions, popular talks and publication of pamphlets in English and local languages.

The following items of the agenda which read as under were deferred for consideration till the next meeting of the Nutrition Advisory Committee :—

(a) To consider whether the recommendation of the Nutrition Advisory Committee, "that the fullest possible use of ground-nut cake flour should be made provided it is prepared under controlled conditions and every care is taken to prevent rancidity" should be modified. (Copy of a letter No. BC 2082/36/NAC, dated the 15th January, 1947, from Professor V. Subrahmanyan, D.Sc., F.R.I.C., Professor of Biochemistry, Indian Institute of Science, Bangalore, to the Secretary, Indian Research Fund Association, and of a letter No. 23/40/46-R, dated the 12th February, 1947 from the Secretary, Indian Research Fund Association, to Professor V. Subrahmanyan, on the subject, are submitted in this connection for the information of the Nutrition Advisory Committee).

(b) To note section 3 (e) of the minutes of the sixth meeting of the Medical Research Liaison Committee for India held in the India Office, London, on the 13th December, 1946.

(c) To discuss the nutritive value of common Indian sweets.

LEPROSY.

1. Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta.

The work during the year included (i) an investigation of the therapeutic value of the sulphone drugs in leprosy, (ii) attempts to culture the leprosy bacillus by Row's method, (iii) a study of the eye lesions in leprosy, and (iv) a study of the histopathology of selected cases of leprosy.

The Sulphone Drugs in Leprosy.

Recently, certain derivatives of diaminodiphenyl-sulphone have been reported on favourably in the treatment of leprosy. These drugs include promin (Parke, Davis & Co.), diasone (Abbott Laboratories) and sulphetrone (Burroughs Wellcome). The matter has been investigated in patients of leprosy of the lepromatous type, and in experimental rat leprosy.

A. Results in Patients.

Selection of patients.— About 50 cases of the lepromatous type, in three groups, have been treated with the three sulphone drugs, promin, diasone and sulphetrone. All the patients were bacteriologically postive and leprominnegative. Most of them had the disease in an advanced stage. Several of them were suffering from complications such as ulceration, eye lesions, repeated reactions, etc. Some of the patients were included for treatment with these drugs specially because they could not stand even small doses of the hydnocarpus oil, and had practically been without any treatment. All the treated patients have been in-patients in the Albert Victor Hospital for Leprosy.

Before starting the treatment, blood examination was made in each case, this included total r.b.c. count, total w.b.c. count, Hb estimation and determination of cell volume. If the red cells and hæmoglobin were found appreciably below normal, the patient was first put on a course of iron with or without liver and the treatment was not started till the blood picture improved. During the course of treatment periodic blood examinations are made and iron with or without liver is given whenever indicated; this was required in about $\frac{3}{4}$ of the cases. In addition vitamin B is given to all the cases who also receive supplementary diet in the shape of increased amount of meat and milk.

In addition to the routine blood examination, the concentration of the drugs in the blood of the patient is estimated periodically.

Doses. —The dose of promin was started at 1 g. and was slowly increased to 5 g. a day, but the maximum could not be reached in all the cases. The dose of diasone was started with 1 capsule ($\frac{1}{3}$ of a g.) a day and has, in most cases, been increased to 4 capsules ($1\frac{1}{3}$ g.) and, in some cases, to 6 capsules (2 g.) a day. The starting dose of sulphetrone was $1\frac{1}{2}$ g. and it has been gradually increased to 6 g. in most cases. All the three drugs in the above doses have been given in courses of 2 weeks followed by one week's rest.

Blood findings.—In almost all cases the administration of the drugs gives rise to lower red cell count and hæmoglobin values, but in most of them the values can be maintained at a fair level by the administration of iron, alone or with liver.

The concentration of the drug in the blood naturally varies with the method of administration. In case of promin, which is given intravenously, the highest concentration has been 10 mg. to 12 mg. per 100 c.c. of blood after a dose of 4 g. to 5 g. and it is reached about an hour after the administration of the drug; in no case has there been a concentration above 12 mg. In case of diasone and sulphetrone, which are given by mouth, the blood concentrations have been between 0·5 mg. and 3 mg., on the average about 2 mg. per 100 c.c. of blood with the doses used. The concentration has never gone higher than 3 mg. and has been very variable on the same dose. It has been observed that the dose which can give a concentration of between 2 mg. and 3 mg. in the beginning, often fails to give rise to similar concentrations when the patient has been treated for some time. The highest concentration is seen 4 hours after the administration of the drug and usually 24 hours after the last dose, no traces are found but in one or two cases the drug has persisted longer.

Results. —The promin group has now been under treatment for 19 months, the diasone group for 13 months, and the sulphetrone group for 7 months only.

Clinical improvement has been seen in most cases in all the three groups. The improvement consists of subsidence of areas of infiltration, disappearance of small nodules, decrease in size and softening of big nodules (both cutaneous and subcutaneous), and improvement in the eye conditions. Ulcers, both general and nasal, heal up early and do not recur. Tendency to reactions is checked or minimized, the reactions not recurring or recurring less frequently and with less intensity.

Bacteriological improvement has not been so marked, in only two cases have the smears become negative, though in others the number of bacilli is generally less.

The treatment has certain limitations. The drugs had no effect on the nerve pains which were a marked feature in some of the cases under treatment. In such cases the abscesses present on the course of the nerves were also not affected by the treatment. Other neural symptoms such as loss of sensation and deformities, etc., also do not improve.

Conclusions. —A definite improvement has been noticed in most cases treated with the sulphone drugs. These drugs, therefore, mark a definite advance in the treatment of leprosy. They are specially valuable in the treatment of those cases of leprosy which cannot stand the injections of hydnocarpus oil.

However, the improvement has been slow and not as marked as one would have expected after reading the reports of some other workers. Moreover, the drugs have definite limitations in that no improvement is seen in the nerve pains and other neural symptoms. It is also yet early to say whether the improvement will be of a permanent nature.

No serious toxic effects have been noticed except for occasional gastric upset, palpitation and feeling of fatigue and weakness specially when higher doses are given. In one case in the promin group signs of nephritis developed during treatment. The drugs have a definite toxic effect on the blood, but in most cases the red cell count and hæmoglobin content can be maintained at a satisfactory level by the administration of iron, alone or in combination with liver. The treatment should, therefore, be regulated by periodic examinations of blood.

The concentration of the drug in the blood on the doses used has never been found to be beyond 12 mg. per 100 mg. per 100 c.c. in cases of promin and beyond 3 mg. for 100 c.c. in the case of diasone and sulphetrone. It is, therefore, concluded that although blood concentration levels may be estimated in cases under experimental treatment, this procedure is not essential and that the treatment can very well be carried on in cases where facilities are not available for estimation of blood concentration.

B. Results in Rat Leprosy.

Batches of white rats, experimentally infected with rat-leprosy bacillus by inoculation of 1 c.c. each of the rat-leprosy suspension, were treated with promin, diasone and sulphetrone. The treatment was started immediately after the inoculation of the rat-leprosy suspension and was continued till the animal died. The longest period of treatment was 5½ months. Doses varying from 30 mg. to 50 mg. to start with were given and were increased to a maximum of 120 mg. a day. The drug was given by means of intraperitoneal injections. It was found that none of the sulphone drugs had any effect in modifying the development and course of experimental rat leprosy in the doses given.

Attempts at Cultivation of the Leprosy Bacillus.

Dr. Row had recently reported successful cultivation of the leprosy bacillus in symbiosis with the leishmania culture. For making the primary cultures he used hæmoglobinized saline, a medium previously successfully used by him for the cultivation of leishmania. The growth from this primary culture is planted on the surface of solid media, hæmoglobinized saline agar and glycerinated potato. Dr. Row reported to have obtained pure cultures of the leprosy bacillus by this method.

Attempts have been made to repeat Dr. Row's work and for primary cultures use has been made of hæmoglobinized Ringer's saline in addition to the hæmoglobinized saline as recommended by Dr. Row. This was done because it was found that the growth of leishmania was better in the Ringer's saline than in the other saline. In all 12 series of 12 tubes each of the fluid media were inoculated with leprous suspension and leishmania culture. While the leishmania was found to grow in most cases, there was no such evidence of the growth of the leprosy bacillus. No doubt that in a few tubes there were some indications of the growth of the bacilli, such as the finding of bunches of bacilli in a few fields, but these were always in association with tissue matter and repeated examinations from the same tube did not constantly show their presence. Moreover, when subcultures were made from these tubes on to solid media, no visible colony or microscopic evidence of growth of the acid-fast bacilli was found. In some cases, however, there was growth of contaminated organisms. Thus, the report of Dr. Row regarding the successful cultivation of the leprosy bacillus in symbiosis with leishmania has not been confirmed.

Eye Lesions in Leprosy.

The eye lesions in leprosy are, broadly speaking, of two different kinds corresponding to the two main types of the disease. There are indications that in India serious eye conditions are much less common than in some other countries. The question has, however, not been studied in detail. It was,

therefore, planned to make a detailed study of the eye lesions in leprosy in collaboration with Major Somerset, Professor of Ophthalmology, Medical College, Calcutta.

Over 200 cases have so far been examined; the study included examination of cornea with a loop and a slit microscope. Only those cases have been included which showed lesions of leprosy on the face and in the neighbourhood of the eyes, since these are the cases most likely to show marked changes in eyes. The findings made so far are summarized below:—

The neural cases.—One hundred and sixteen cases of the neural type have been examined, 92 bacteriologically negative, and 24 positive. In about half the cases, the disease was of slight extent (Nml), and in other half of fairly marked extent (Nm2). All the cases had lesions on the face and in 72 cases the eyelids were invaded on one or both sides.

About half the cases complained of some eye symptoms, in most instances burning, pain or watering, a few complained of dimness of vision, and only in two was there complete loss of sight in one eye.

The eyebrows showed a slight loss on the outer side in less than one-third of cases, and slight loss of eyelashes in a few cases.

In 12 cases there was weakness in the lid movements, in 7 the weakness was marked enough to cause lagophthalmos. The lagophthalmos was always associated with conjunctivitis, loss of corneal sensation, and exposure keratitis. Another condition resulting in difficulty in closing the eyelids and consequent exposure keratitis, etc., is the presence of thick swollen lesions on both the eyelids during the phase of reaction. This condition is possibly not described in literature and is not caused by any weakness in the orbicularis oculi muscle, or by inflammation and hardness on account of turgescence. In this condition the lids are immobilized in the open position and are so hard, tense and infiltrated that they cannot be closed either by the efforts of the patient or by the fingers of another person. The cornea thus remains exposed, dries up and ulcerates; secondary infection rapidly supervenes sometimes with the total loss of vision and often of the whole eye. Two such cases were seen during the course of the study.

About one-third of the cases showed signs of conjunctivitis, not specific of leprosy. In no case were there any signs of episcleritis.

Lesions in the cornea are not frequent; when present they are of the nature of haziness of cornea, exposure keratitis, and resulting ulceration. The lesions are more or less confined to the lower half of the cornea. Marked ulceration with destruction of whole cornea resulting in loss of sight was seen in 2 cases.

The lepromatous cases.—Eighty cases of the lepromatous type have been examined; in 58 the disease was fairly extensive (L_2), and in 22 it was advanced (L_3). In all the cases there were lesions on the face and the eyelids.

Two-thirds of the cases complained of eye symptoms. In most instances there was pain, watering, photophobia, etc., in 17 there was dimness of vision, and in 2 complete loss of sight.

In three-quarters of the cases there was marked loss of brows, specially on the outer side, in several cases the brows were completely lost. There was slight to marked loss of eyelashes in about half the cases.

Weakness of the lid movements was seen in only five cases being slight in 4 and marke d in 1case.

Signs of episcleritis were found in 16 cases and another 21 suffered from conjunctivitis. Smears taken from conjunctiva in a small number of cases showed the presence of leprosy bacilli.

Leprous lesions were found in the cornea in about half the cases, in most instances the lesions were confined to the upper and outer parts of the cornea. The lesions were of the nature of either superficial punctate keratitis, or interstitial keratitis. In one case leprous nodules were seen in the cornea at the limbus.

The superficial punctate keratitis is in many instances pathognomonic. It is situated in the upper and outer quadrant of the cornea spreading from the limbus for a distance of some 2 or 3 millimeters. The individual punctate spots look like "grains of chalk". Vascularization is either absent or extremely slight—quite a different picture from trachomatous pannus. The condition is confined to the epithelium.

Two types of interstitial keratitis occur. The most common is a grey infiltration of the substantia propria spreading from the limbus of the outer and upper quadrant of the cornea and usually associated with an adjoining episcleritis. It appears to be an extension of the inflammation of the episclera into the cornea. Deep vascularization is usually absent as is also iritis.

The other and much rarer involvement of the deeper layers of the cornea is a deep punctate keratitis. Small grey dots about 1 mm. in diameter are seen scattered in the substantia propria—particularly in the upper and outer quadrant. They are unassociated with vascularization or thickening of the corneal nerves.

Signs of present or past iritis were found in about 30% of the cases, nodules on the iris were found in 4, and in 9 the pupil was small and sluggish.

Conclusions.— The study supports the belief that serious lesions of eyes in cases of leprosy are much less frequent in India than in some other countries. For example, Lopez (1890) in Havana, Santonastaso (1931) in Italy and Poole (1934) in Honolulu found ocular involvement in every case. Harley (1946) reported a study of 150 cases of leprosy, and concluded that in Panama 90% of the leprous patients were found to have ocular involvement, the 10% of eyes free from the disease were found exclusively in recent cases. In this series of 150 cases, 13% were totally blind in one or both eyes, 51% had markedly defective vision, and in only 43% was the vision normal.

Further, the study confirms the view that the eye lesions in leprosy are of two different kinds, corresponding to the two main types of the disease.

In the neural type the lesions are secondary to the nerve involvement; the most marked lesions are the weakness of the orbicularis oculi muscle, loss of sensation of the cornea, and the resulting exposure keratitis, in some cases leading to ulceration of cornea. Similar lesions of the cornea may be produced by thick and swollen lesions on the eyelids resulting in difficulty in bringing the two lids together. In the neural type there is no involvement of episclera and iris, altough in a few cases there is secondary iritis from corneal ulceration following on exposure keratitis.

In the lepromatous type, there is actual leprous infiltration of the eye tissues, both superficial and deep. There may be weakness of the lid movements, and a little loss of sensation of the cornea in some cases, but these are not marked features. The most marked lesions in this type are leprous episcleritis, superficial punctate and deep keratitis, and iritis.

Histological Studies.

The histological findings in the typical lesions of the two types of leprosy are well recognized. In some cases certain histological features are observed which are not typical of either type and some workers have attached special significance to these features. However, the significance attached to these features is a disputed matter. The best method of arriving at sound conclusions would be to correlate these atypical histological features with other findings in these patients, specially the immunological findings and the subsequent course of the disease. This is being attempted and a fairly large number of cases has now been under observation for a considerable period. In these cases repeated bacteriological, immunological, histological, and clinical examinations have been made. These cases include amongst others two groups of cases: (i) cases of doubtful classification which would not be classified as neural or lepromatous on clinical grounds, and (ii) cases of tuberculoid reaction which during the phase of reaction have certain atypical clinical and histological features. The findings in these two groups of cases are briefly analysed below :—

1. *Cases of doubtful classification.*—By keeping these cases under prolonged observation, it has been possible to sort them out into three groups :—

(i) In about half the cases the subsequent progress of the disease has been similar to that of cases of the lepromatous type, i.e. the disease has progressed gradually and has been active over a long period. In these cases the lepromin test has always been negative except in one case in which the results of the test have been variable, being positive in the stage of increased activity and negative at other times. The histological findings have been those of the lepromatous type, but in two cases definite tuberculoid foci were seen. These cases are considered to be really lepromatous and it is considered that localized lepromatous lesions occur more frequently than is generally recognized. The study moreover shows that the existence of tuberculoid foci in a lepromatous lesion is not of much significance.

(ii) In a small number of cases the subsequent progress has been like that of the tuberculoid lesion, i.e. early subsidence of the activity, although in a few this was later followed by a reaction, but which also subsided earlier. The lepromin test has always been positive, the histological picture was that of a tuberculoid lesion although there were present atypical features such as marked vacuolation of some cells, scanty or small giant cells, and little affected nerves inside the foci of granuloma. These cases are considered to be really tuberculoid during the phase of reaction ; similar histological findings have been made (*vide* below) in recognized cases of tuberculoid reaction.

(iii) In the remaining cases the subsequent course of the disease has not been clear cut and has not conformed to the course in the recognized types. The lepromin test has given variable results and the histological findings have also been "doubtful". In these cases the doubt in classification has not been cleared up even with the help of repeated histological and immunological examinations. Perhaps they may form a class by themselves.

2. *Cases of tuberculoid reaction.*—In this group are included tuberculoid cases in which the history of the disease definitely indicated the presence of

reaction, i.e. the disease had existed for some time, but the signs of exacerbation were present only for the previous few months. Clinically the lesions in some of the cases were typically tuberculoid with well-defined margins in others the lesions were smooth with a shiny surface and ill-defined margins.

As a result of study these cases could be divided into three sub-groups :—

(i) *Cases with lepromin test always positive.*—In this group there was always early subsidence although in a few there was relapse of activity (again followed by early subsidence). Bacteriologically these cases were either always negative or slightly positive in the beginning and became negative with the subsidence of the disease. Histologically the picture was that of a tuberculoid lesion although there were atypical features such as the presence of highly vacuolated cells, scanty giant cells, slightly infiltrated nerves inside the foci of granuloma and, in a few cases, a clear sub-epidermal zone. With the subsidence of the disease, however, the histological picture changes and we see usually the changes found in a " simple " lesion. Judged by immunological, bacteriological and clinical criteria these cases could not but be accepted as typical tuberculoid cases. The histological findings made in these cases including the so-called atypical findings have, therefore, to be considered as featues of tuberculoid histology specially present during the phase of reaction.

(ii) *Cases with variable lepromin reaction.*—Clinically these cases also showed early subsidence but they usually had repeated reactions, bacteriologically they were slightly to moderately positive in the beginning but became negative with the subsidence of the lesions. Histological picture was that of a tuberculoid lesion with the special features described above. It is possible that a further follow-up of these cases may indicate that these cases differ from cases in the first group.

(iii) *Cases with negative lepromin.*—Amongst the cases clinically recognized as cases of tuberculoid reaction there was a small number in which the clinical progress of the disoase was later found to be similar to cases of the lepromatous type, the disease remaining active and bacteriologically positive over a long period. The histological picture was that of a lepromatous lesion although at places there were suggestions of tuberculoid arrangements. The findings in these cases again indicate that the presence of tuberculoid foci in an otherwise lepromatous histology is not of much significance.

2. Study of the histopathology of leprosy under Dr. R. G. Cochrane at the Christian Medical College, Vellore.

The work of this enquiry has been of a preliminary nature because of the following reasons :—

(i) The inability to recruit the full staff sanctioned in this enquiry by the Indian Research Fund Association in April last.

(ii) Owing to the officer in-charge of leprosy enquiry being away in the United Kingdom on a special deputation for 2½ months.

The steps of the enquiry were laid down last year under the following heads :—

(i) Review of the work already done in the light of our present knowledge of the cellular reaction in leprosy with particular reference to tissue-defence.

(ii) A study of all records with a view to correlating available material and discarding that which is of little value, summarizing the research done up to date.

(iii) When the general picture of tissue reactions in leprosy has been worked out particular attention will then be paid to further research along the following lines.

A. *Histopathological changes in lepromatous leprosy particularly in relation to.*—

(i) The significance of the sub-epidermal free zone so frequently seen in this condition.

(ii) A study of the nerves in leproma. These show no cellular infiltration but there are undoubtedly pathological changes in the individual bundles.

(iii) Further investigation into leproma to see whether there are different varieties which can be determined by histopathological changes in the skin, and if they are the prognostic significance of these varieties.

(iv) Detailed investigation of acid-fast-bacilli in the skin in leproma of various degrees of advancement, where the *M. leprae* life in relation to the macrophages and their distribution throughout the corium of the skin.

B. *Transitional forms of leprosy.*— This large group of cases furnishes a very interesting pathological picture and particular emphasis will be placed on

(i) The significance of the large capillaries in the free sub-epidermal zone so aften seen.

(ii) The significance of giant cells associated with foamy cells.

(iii) Detailed study of sarcoidal leprosy.

C. *Simple macular.*— Special attention will be paid to the changes in the nerve-terminals in the skin in relation to

(a) Hypopigmentation. (b) Anæsthesia.

D. *Neural anæsthetic leprosy.*— Studies into the question whether in neural anæsthetic leprosy there is a true peripheral neurities (an interstitial fibrosis of the nerve-terminals).

E. *Animal experimentation.*—This is to be continued in the light of the results during the past 6 years. A summary of this investigation is now in the press and will be published in the *International Journal of Leprosy.*

It is hoped that this type of research will lead to further lines of investigation and it cannot be undertaken without the co-operation of a full time research worker trained in pathology. Further, along with this research a certain amount of work on the bacteriology of the disease may have to be undertaken.

This work will be done in co-operation of the King Institute, Guindy and the Pathology Department of the Christian Medical College, Vellore.

During the year the material available has been reviewed and sections of the various types of lesions with clinical photographs are being collected and this will form a useful basis for the development of future work. Various special techniques were experimented with, especially in relation to the demonstrating nerve-tissue in sections and the staining of bacilli especially in Zenker fixed material. Encouraging progress has been made in this respect.

It is hoped that we shall shortly have the technique of staining acid-fast bacilli in tissue so perfected that it will be possible to demonstrate acid-fast bacilli in lesions of neural leprosy and in the nerves. The late Professor H. P. Lie of Norway maintained that it should be possible to find acid-fast bacilli in hypopigmented lesions of neural leprosy. In fact he demonstrated to the officer in-charge of the enquiry some years ago that he could find acid-fast bacilli in a lesion in which bacilli were not found by standard methods of examinations. The significance of developing this work cannot be exaggerated. With regard to the demonstration of nerve-tissues the officer in-charge of the enquiry is in touch with Dr. Weddell of Oxford University who has promised to give all assistance possible.

Experiments have been continued on the newer drugs in leprosy, viz. promin, diasone and sulphetrone. These remedies are being investigated in the light of the knowledge of the pathology of the disease. It is hoped that the work being undertaken will lead to more practical methods of administration of the sulphone group of drugs.

When the full personnel of the enquiry has been appointed it is expected that considerable progress in the above directions will be made.

Mr. J. C. Lawrence, Halley Stewart Trust Research Fellow has shown great skill in his technique and has greatly assisted in the work. Mr. Paul, technician under the Director, Leprosy Campaign, Madras Presidency, has also assisted in the work.

List of papers published.

CHATTERJI, S. N., and DHARMENDRA.—Legopthalmos in Leprosy (*Leprosy in India*, **19**, No. 2).

DHARMENDRA, and MUKERJI, N.—Lepromin Reaction in Subsided Lepromatous Cases (*Leprosy in India*, **19**, No. 1).

DHARMENDRA, and KAIKARIA, S. S.—Failure to Sensitize Presumably Non-leprous Individuals to Lepromin (*Leprosy in India*, **19**, No. 1).

DHARMENDRA, and MUKERJI, N.—Treatment of Leprosy with Hydnocarpus Remedies (*Leprosy in India*, **19**, No. 2).

DHARMENDRA.—The Lepromin Test—a review (*Leprosy Review*, Lond.). (*in Press*).

DHARMENDRA.—Leprosy in Ancient Indian Medicine (*International Journal of Leprosy*). (*in Press*).

REPORT OF THE MEETING OF THE LEPROSY ADVISORY COMMITTEE HELD AT 2-30 P.M. ON THE 10th DECEMBER, 1947 IN THE IMPERIAL SECRETARIAT, NEW DELHI.

PRESENT.

Dr. C. G. Pandit. (*Chairman*).
Lieut.-Colonel A. N. Chopra.
Dr. P. V. Gharpure.
Dr. V. R. Khanolkar.
Dr. R. G. Cochrane.
Dr. Dharmendra (*Secretary*).

IN ATTENDANCE.

Dr. S. W. Hardikar
Dr. B. B. Dikshit
Dr. S. R. Pandit
Dr. M. N. Lahiri.
Lieut.-Colonel B. N. Hajra.

1. The progress of work on the leprosy enquiries under Dr. Dharmendra at Calcutta and Dr. Cochrane at Vellore, was considered. After discussion it was decided to recommend the continuance of the two enquiries on the lines indicated in the proposals for next year. It was decided to request Dr. Cochrane and Dr. Dharmendra to prepare a proforma for recording the data of trials of chemotherapeutic compounds used in the treatment of leprosy and relevant laboratory findings obtained during the course of such trials.

2. The following papers were considered and recorded :—

(a) Section 2 (vi) of the minutes of the 7th meeting of the Medical Research Liaison Committee for India, held at the India Office, London, on the 20th March, 1947.

(b) Comments by Dr. Ernest Muir on the report of the meeting of the I.R.F.A. Leprosy Advisory Committee, held at the Haffkine Institute, Bombay, on the 12th December, 1946.

(c) A preliminary report by Dr. L. H. Wharton Mahaica Leprosarium, British Guiana, on the use of sulphetrone in the treatment of leprosy.

3. The following items on the agenda were deferred for future consideration :—

(a) The review of lepromin test by Dr. Dharmendra.

(b) The co-option of a member on the Committee for review of leprosy work in India.

PLAGUE.

1. Plague researches under the Director, Haffkine Institute, Bombay.

I. Plague Vaccine.

(a) On the analogy of the enhancement of immunogenic effect by the addition of alum (potash) to toxoids, experiments were tried to study whether a similar effect is noticeable in the case of vaccines treated with alum. A fair number of experiments were carried out with various concentrations of alum, beginning with small doses and reaching a concentration enough for full precipitation. The following results were obtained :—

	50% mouse-protective dose, in c.c.
(1) 2 weeks' vaccine grown at 28° C. in casein hydrolysate, killed with 0·1% formalin and preserved with 1.5 mg. phenyl mercuric nitrate per 100 c.c. ..	0·0034
(2) Vaccine No. 1 (above) with 0·1% alum ..	0·0040
(3) Vaccine No. 1 (above) with 0·3% alum ..	0·0046
(4) Vaccine No. 1 (above) with 0·7% alum ..	0·0037

(b) *Casein hydrolysate medium.*— The casein hydrolysate prepared by digesting casein with hydrochloric acid and sulphuric acid gives a very dark-coloured medium. An attempt was made to decolorize the medium. A large number of samples of activated charcoal were employed for the purpose. After great deal of experimentation, it was found that Nuchar brand of charcoal (No. C-190 NU) gave the best results. It produced a very clear medium with merely a slight tinge of yellow. The original medium, as prepared without decolorization with charcoal, gave a growth of about 360 million organisms per c.c. and to our utter surprise, it was found that after the medium had been decolorized with Nuchar, it gave as many as 700 to 800 million organisms per c.c.

This is an observation of great biological interest. The expectation was that decolorization would possibly reduce the nutrient property of the medium. When a reverse effect was, however, observed, the question immediately arose as to the nature of the substance that prohibited growth. The work done so far shows that it is very likely of the nature of an amino-acid. Active work is proceeding to identify and specify the nature of the anti-growth factor.

II. Chemotherapy of Plague.

Curative value of streptomycin in experimental plague infection. —Following numerous reports about the excellent curative properties of streptomycin against various Gram-negative bacilli, experiments were conducted to assess the curative value of the drug in experimental plague infection in mice. Treatment with the drug was started (1) 48 hours after infection when nearly 50·0% of the animals show septicæmia, (2) 72 hours after infection, when 100% of the animals show septicæmia.

The following results were obtained :—

Drug given subcutaneously ; requisite quantity of the drug contained in 0·2 c.c. of the fluid.

Drug administration started 48 hours after infection.

Dose. (1)	Number of deaths in test animals (2)	Number of organisms in challenge dose. (3)	Deaths in controls. (4)
0.2 mg. four times daily for 7 days ..	*3/10	5	*12/12 †(5·6)
0.4 mg. four times daily for 2 days followed by 0·2 mg. four times daily for the next 5 days ..	0/10	136	10/10 (4·9)
0.4 mg. four times daily for 2 days followed by 0·2 mg. four times daily for the next 5 days ..	1/10	124	10/10 (6·0)
Drug administration started 72 hours after infection.			
0·2 mg. four times daily for 7 days ..	5/10	140	12/12 (5·1)
0·8 mg. four times daily for 2 days followed by 0·2 mg. four times daily for the next 5 days ..	1/10	124	10/10 (6·0)
Drug administration started 48 hours after infection. Drug administered either in one or two large doses only.			
4·0 mg. followed by another dose of 4·0 mg. 48 hours later ..	4/5	95	12/12 (5·6)
4·0 mg. in one dose only ..	9/10	136	10/10 (4·9)
Drug administration started 72 hours after infection. Drug administered in two large doses only.			
4·0 mg. followed by another dose of 4·0 mg. 48 hours later ..	5/5	140	12/12 (5·1)

* The upper figures of columns 2 and 4 denote the number of deaths and the lower figure the total number of mice used.

† The figure in brackets in column 4 denotes the average duration of life of the controls.

III. Field Trials with Sulphamerazine.

A plague epidemic at Poona during the latter part of 1946 and early part of 1947, gave an opportunity to conduct a trial of the comparative curative value of sulphadiazine and sulphamerazine in the treatment of bubonic plague. The trial was carried out in the Infectious Diseases Hospital at Poona and lasted from the beginning of March to the 25th of April, 1947.

The drugs were given in the following dosage :—

Sulphamerazine, up to case No. 43, an initial dose of 4·0 g. was given followed by 2·0 g. four hours later. Then 1·0 g. was given every eight hours. The patient in this way received 8·0 g. of the drug on first day and 3·0 g. a day on subsequent days. This dosage gave a blood concentration of 15·0 m.g. to 20·0 mg. per cent and over in majority of the cases and since this concentration was considered to be slightly higher than necessary, from case No. 45, the sulphamerazine patients received as their second dose, 1·0 g. instead of 2·0 g., other doses remaining the same. These patients thus received 7·0 g. on the first day instead of 8.0 g. This slight reduction in dosage did not, however, change the blood concentration materially.

Sulphadiazine was given in an initial dosage of 4·0 g. followed by 2·0 g. four hours later. Thereafter 1·0 g. was given every four hours. The patients thus received 10·0 g. on the first day and 6·0 g. a day on subsequent days. This dosage gave a blood concentration of 10·0 mg. to 15·0 mg. per cent in approximately half the number of cases and of 15·0 mg. to 20·0 mg. per cent and over in the remaining ones.

Both the drugs were, as far as possible, given orally. Cases which were comatose or were unable to swallow the drugs for any reason received the drugs intravenously or intramuscularly. The level of the drug in the blood was estimated once daily at a fixed time in the morning. The drugs were given, if necessary, for a total period of 10 to 12 days, but were usually stopped two days after the temperature reached normal.

Results.—Of the 107 cases admitted into the hospital, 100 cases subsequently turned out to be plague. From these, 19 cases are excluded from reckoning, (1) 5 convalescent cases, (2) 12 moribund cases, (3) one case left hospital against medical advice, (4) one case given combined treatment with anti-plague serum and sulphamerazine.

Results obtained from the remaining 81 cases are summarized in the following tables :—

Treatment	Number of cases	Number of deaths	Case mortality, per cent
Sulphadiazine	40	4	10·0
Sulphamerazine	41	3	7·3

Since septicaemic cases give a better idea of the curative power of any treatment, the results of septicaemic cases are given below separately.

Treatment	Number of cases.	Number of deaths	Case mortality, per cent
Sulphadiazine	7	2	28·5
Sulphamerazine	9	2	22·2

No serious toxic symptoms were seen in any of the cases treated with the two drugs. The results do not show any significant difference between the two types of treatments, but the number of cases treated is very small to draw any definite conclusion. From the results, it appears that sulphamerazine is the drug of choice, as it maintains with lesser dosage as high a blood concentration as sulphadiazine and what is more is that it requires less frequent administration.

2. Plague enquiry in the Nilgiris district under the Director of Public Health, Madras.

Plague enquiry in the Nilgiris was continued during the year along with the Government scheme for eradication of plague. The materials collected during the routine working of the plague control operations, were utilized for research purposes at the Central Laboratory located at Coonoor.

General.

Plague has been endemic in this district for over four decades. As compared to the endemic areas in the plains, epidemiological features such as the high average altitude, temperate climate prevailing throughout the year, large variety of rodents and fleas, special housing conditions, insignificant traffic in grains, cotton, etc. the net work of plantations of tea, coffee and rubber and the extensive cultivation of potatoes in close proximity to dwellings, are peculiar to this district. Apart from the rat-flea surveys carried out in 1929 in just a few selected places and the short study of sylvatic plague undertaken in 1939, very little information is available of the prevailing factors contributing to the endemicity of plague here. The field of this enquiry, therefore, presents valuable opportunities in the elucidation of numerous problems connected with the recrudescence of plague infection, the elimination of the nuisance of the prolific field rodents and special measures required to control plague in hill tracts.

Plague.

During the year under report Ootacamund and Coonoor Municipalities were free from plague. In the rural areas of the district there were 55 attacks and 13 deaths reported, from 38 villages and hamlets up to the end of September 1947 compared to 83 attacks and 38 deaths in 41 villages and hamlets in the year 1946. There were 19 more villages where infection abruptly stopped at the epizootic stage owing to prompt preventive measures which were undertaken.

Progress of Work.

1. *Cyanogas fumigation.*— 38 villages where indigenous human cases had occurred and 19 villages soon after onset of rat falls, were fumigated with cyanogas. Infected buildings were generally fumigated in toto, while only the rat-burrows, cracks in walls and such other obvious rat harbourages were fumigated in the other buildings. All infected villages were fumigated at frequent intervals according to the nature of the infection. Fresh human cases occurred only in two of the villages in the first group and rat plague was aborted quickly in all the villages in the 2nd group as a result of these fumigation.

Villages with a history of repeated epidemics in the past and those which were specially threatened this year were fumigated once every 3 months. Villages which had only sporadic infection in the past were fumigated at wider intervals of 4 to 5 months. All these villages escaped plague this year.

In the Municipal towns of Ootacamund, Coonoor and Kotagiri the market stalls, grain godowns, potato stores, grocery shops, etc., were fumigated regularly every month. No plague occurred in any of these towns this year.

2. *Rodents and their fleas.*— Rodents obtained during the fumigation operations and those specially caught from the fields were first searched for fleas and then dissected for examination for plague infection.

The chief rodents concerned with the spread of plague in this district are the house rats (*R. rattus*), banticoots, (*B. malabarica*), mole rats (*Gunomys kok*), house mice and field mice. The fleas on the house rats were chiefly *X. cheopis* (46·5%) and *X. brasiliensis* (36·5%) with small numbers of *Ceratophyllus, Stivalius, Leptopsylla,* and *Pulex irritans.* On the mole rats, the chief infesting fleas were *Stivalius* (69%), *X. cheopis* (12%) and *X. brasiliensis* (12%) with small numbers of *Ceratophyllus.* The fleas on house mice were largely *Barsiliensis* (35%), *Ceratophyllus* (30·5%), *Cheopis* (24%) and *Leptopsylla* (10·5%).

3. *Studies in sylvatic plague.*—The abounding mole rats in potato fields present a major problem in Nilgiris, both in the field of agriculture and in that of plague control. These rats are known to swarm into houses during the off-season of potato cultivation while they keep to the fields at other times. They are susceptible to plague and carry fleas which are efficient vectors of plague. The role of these rodents and fleas in the recrudescence of plague has been taken up as a special subject for study. Two hundred and twenty-nine mole rats and fifty-nine field mice were caught and 429 fleas collected from them. The rodents and fleas were both examined for plague infection. The results were all negative. This investigation will be continued with specimens from other areas and with special reference to the occurrence of plague in the vicinity.

4. *Studies on the use of DDT in plague control.*—Two small villages about 2 miles apart containing about 500 houses each were selected for experiment and control. A rat-flea survey was carried out in April 1947 in both these villages, before the commencement of the experiments. The general flea indices were 3·1 in the experimental village and 3·6 in the control village. Neocid powder (10% DDT) was used in these experiments. The dusting of Neocid, in the rat-burrows was done with the help of cyanogas foot-pumps. Sufficient quantity of the powder was pumped in, till it was seen to emerge as a white cloud from the connecting outlets. These outlets were then closed with mud packing and some more powder blown in. Air alone was then blown in to ensure even distribution of the powder in the burrows. Finally, the mud packings were removed and the openings of the burrows left open. All rat-burrows, cracks in walls, firewood and stone heaps, collections of sundry articles were all dusted with Neocid. A few locked up houses in the village could not, however, be dealt with. Systematic rat-trapping was done in every house in the two villages every month after the commencement of the experiment, to procure rats to study the extent of their flea infestation. The rat-flea indices for July and September 1947 in the control village were 3·6 and 5·6 respectively. The corresponding indices for the experimental village were 0·65 and 0·3 respectively. These results are very encouraging. The observations in these villages will be continued to decide how long the effect of Neocid will last.

The reduction in the nuisance from *Pulex irritans* on the floors of houses aftrer dusting with Neocid was observed in earlier experiments.

REPORT OF THE PLAGUE ADVISORY COMMITTEE.

The Committee met at 10-00 a.m. on the 10th December, 1947, in the Imperial Secretariat, New Delhi.

MEMBERS PRESENT.

Major-General Sir Sahib Singh Sokhey. (*Chairman*).
Dr. P. V. George.
Dr. A. N. Goyle.
Dr. V. R. Khanolkar.
Dr. R. B. Lal.
Dr. C. G. Pandit.
Lieut.-Colonel S. D. S. Greval. (*Co-opted*).
Dr. K. V. Venkatraman. (*Secretary*).

Dr. M. Sharif and Dr. P. M. Wagle could not attend.

The following also attended :—

Colonel A. N. Chopra.
Dr. S. R. Pandit.
Dr. C. O. Karunakaran.
Lieut.-Colonel C. Mani.
Lieut.-Colonel A. N. Duggal.
Dr. M. O. T. Iyengar.
Dr. D. K. Viswanathan.
Dr. B. B. Yodh.
Dr. P. V. Gharpure.
Dr. A. R. Mehta.
Dr. V. N. Patwardhan.
Dr. M. L. Bhatra.
Dr. I. M. Puri.

1. *Plague Enquiry under the Director, Haffkine Institute, Bombay.*

(a) *Plague vaccine.*—Major-General Sir Sahib Singh Sokhey presented the results of experiments which were carried out with vaccines treated with alum with a view to ascertain if a single dose of alum-precipitated vaccine would give the same degree of immunity as the untreated vaccine given in two or more doses. It was expected that alum-treated antigen would remain at the site of inoculation for a longer period and would thereby increase the immugenic value of the vaccine. The results of experiments did not bear out this expectation.

(b) *Casein-hydrolysate medium.*—Casein-hydrolysate prepared by digesting casein with hydrochloric acid and sulphuric acid gives a very dark medium. An attempt was made to decolorize the medium and for this purpose Nuchar brand of charcoal was found to be most effective. There was reason to believe that this treatment would possibly reduce the nutrient property of the medium but by actual growth determinations it was found that it enhanced the growth. The untreated casein-hydrolysate gave a growth of about 360 million organisms per c.c. but the decolorized medium gave as high growth as 700 to 800 million

organisms per c.c. Attempts were made to isolate this growth-preventing substance and the work so far done seems to indicate that it is of the nature of an amino-acid. Work will be continued to identify the anti-growth factor. Major-General Sokhey also informed the Committee that decolorized medium is likely to yield a vaccine of much greater protective value. Early results show that the protective power is approximately twice that of the present one.

Chemotherapy of plague :

(i) *Experimental.*—Streptomycin in experimental plague infection: Streptomycin was administered to groups of mice 48 hours after infection when nearly 50% of the animals showed septicæmia, and 72 hours after infection, when 100% of the animals showed septicæmia. The results of the administration of 0·4 mg. four times daily for two days in the case of the first group and 0·8 mg. four times a day followed by 0·2 mg. for the next five days in both groups gave very striking results. Nine out of 10 animals survived the challenge dose even in the second group which is a very severe test.

(ii) *Field trials with sulphamerazine.*— On the lines of control experiments presented to the Committee on previous years sulphamerazine and sulphadiazine were tried on alternate cases in an epidemic in Poona. Only 81 cases became available for this control experiment ; this number was not large enough to give statistically significant results. These experiments, however, appeared to show that the therapeutic value of both sulphamerazine and sulphadiazine is equal. Major-General Sokhey pointed out that sulphamerazine had one great advantage over sulphadiazine in that it required to be administered only once every 8 hours as against sulphadiazine which required to be administered every four hours to maintain adequate concentration in blood. He considered that in India this factor was of considerable importance. He also told the Committee that he has just started a field trial for assessing the value of streptomycin in the treatment of plague. In the few cases treated so far, the results seem to be very striking. The trial will be continued to get an adequate number of cases.

In the discussion that followed Dr. Khanolkar suggested that in view of its importance, a histological study of viscera of mice surviving after streptomycin, treatment may be undertaken to have the results compared with treatment with sulpha drugs. Major-General Sokhey welcomed this suggestion and undertook to make the observations.

Dr. Lal pointed out that there was considerable difference in the mortality rate in Bombay and the C.P. and desired to know if it would be possible to conduct these trials in the C.P. He also referred to the question of toxicity of streptomycin and desired to know if this has been observed. Major-General Sokhey expressed his willingness to make trials wherever facilities are provided but he pointed out that in the areas where he is working now the mortality is very high and that should yield convincing results. It was his impression that it had been stated that mortality in the C.P. was lower than in other provinces.

(iii) *Standardization of the dose of plague vaccine.*— Dr. Wagle found that in the few cases that he could get, serum from human cases convalescing from plague in 1-c.c. amounts was mice enough to protect five out of six mice infected with 10 MLD of plague culture. His attempt was to see if a similar degree of immunity could be obtained in rabbits and mice by means of immunization of plague vaccine. Early results seemed to show that this is too high a standard to attain. The work is continuing.

The Committee recommended that the enquiries under the Director, Haffkine Institue should be continued and the grant asked for sanctioned.

2. *Plague Enquiry in the Nilgiris under the Director of Public Health, Madras.*

Dr. George described the results of cyanogas fumigation in 38 villages and DDT spraying in two villages. Cyanogas fumigation once in every three months was effective in preventing the outbreak in those villages. For DDT experiments two villages two miles apart containing about 500 houses were selected. A rat-flea survey was carried out in April 1947 before the commencement of the experiment. The general flea indices were 3·1 in the experimental village and 3·6 in the control village. Ten per cent DDT powder was dusted into rat burrows with cyanogas foot pumps. Systematic rat trapping was done in every house in the two villages every month. The rat-flea indices for July and September 1947 in the control villages were 3·6 and 5·6, respectively, and only 0·65 and 0·3 in the treated villages. These results are very encouraging.

Major-General Sokhey expressed satisfaction with the way this work has been done; in that he believed that DDT was likely to be the most important anti-plague measure. Lt.-Col. Mani referred to the experience of French authorities in Algeria where severe plague broke out and DDT spraying proved a very effective anti-plague measure.

The Committee considered the results obtained to be very encouraging and were of the opinion that it should be continued. They recommend that the grant of Rs. 6,000 paid annually to the Madras Government towards research aspect of this investigation should be continued.

Dr. D. K. Viswanathan supplemented Dr. George's observations with data collected in Manchar, a small town in Poona district. In this town DDT spraying was done in rat-burrows of one half of the town and the other half was left untreated. The reduction in the flea index was of the order noticed in the Nilgiris. A few weeks after this treatment had been carried out, plague broke out and in spite of the fact that it must have been possible for rats to migrate from one part of the town to the other yet not a single case had occurred in that part of the town which had been sprayed with DDT while 40 cases occurred in the non-treated part of the town. He also stated that in the extensive malaria control programme in Dharwar and Kanara involving 1,200 villages, where DDT spraying was limited to the walls and roofs of houses, there has not been a single case of human plague in the last 18 months even though there were rat-falls in about a dozen villages.

The Committee were greatly interested in the observations made by Dr. Viswanathan and recommend that the Bombay Government should be requested to continue these investigations and include treatment of rat-burrows with DDT in selected villages in their anti-malaria operations in regions where plague outbreaks are usually experienced and observe the results on the incidence of plague.

3. *New Enquiries.*

Major-General Sokhey described a proposal to conduct an enquiry on "a quantitative study of the nutrition requirements, growth factors and growth inhibitors of the plague bacillus" at the Haffkine Institute, for which a request for a grant of Rs. 13,948 has been made. It was proposed to study the nutritional

requirements of the plague bacillus in synthetic media on a quantitative basis, i.e. by measuring the degree of growth of the bacillus by (i) viable counts and (ii) estimation of the total nitrogen. Parallel to the quantitative estimation of the growth, the protective power of the vaccine produced will be measured in mice.

The Committee considered that an enquiry on the lines proposed would be of considerable value but in view of the present financial stringency they suggested that the request for a grant may be deferred. They, however, suggested that the Director, Haffkine Institute, should consider the desirability of suggesting a candidate for one of the Fellowships of the Indian Research Fund Association and make a beginning by starting him on a study of this problem.

Dr. George presented a report summarizing the work done by the Plague Enquiry in Hosur Taluk during the years 1941-46. The results of fumigation experiments showed that in the villages fumigated immediately following the detection of rat plague the results have been most striking; the epizoötic was immediately aborted. In villages where human infection had just commenced, either no more cases occurred or the epidemic was rendered very mild. In big villages where fumigation was started after the epidemic had been well established the results were rather disappointing. Fumigation of threatened villages has given very satisfactory results in an appreciable reduction in rat density and the rat-flea index. Results of treatment of plague cases with sulphathiazole have been very striking. The Committee resolved to record this very interesting note.

4. An extract of section 2(v) of the minutes of the 7th meeting of the Medical Research Liaison Committee for India, held at the India Office, London, on the 20th March, 1947 was read. The Committee recommended that the Directors of Public Health and the Directors of Laboratories may be addressed on the subject and information received from them on the points mentioned made available to this Committee. With regard to the last suggestion of the Liaison Committee trials in human cases have already been undertaken and the results will be communicated to them when the trials are complete.

CLINICAL RESEARCH.

1. Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

Studies on Experimental Production of Cancer by Feeding P-Dimethylaminoazobenzene.

The study on the experimental production of cancer of the liver in rats by feeding p-dimethylaminoazobenzene (p-DAB) undertaken last year is still in progress. Rats of two different strains, Haffkine and Wistar, were fed a diet consisting of 2 c.c. of 3 % p-DAB per 100 g. rice with 2·0 g. carrot and one fresh green leaf per day. Seven groups of animals of each strain were kept on the experimental diet and each group was sacrificed at an interval of one month from the beginning of the experiment for biochemical and histological study of liver during the course of tumour development. Similarly, control groups on the experimental diet without the carcinogen and on stock diet were sacrificed at the end of three and six months of feeding.

Growth of animals. —Animals of both the strains showed a very slow increase in weight, which was on an average 17 g. per animal during six months of feeding experimental diet with carcinogen. The animals on experimental diet without p-DAB showed an average gain in weight of about 60 g. per animal as against a rise of 100 g. per animal observed in animals kept on stock diet only.

Mortality.—There was no mortality in Wistar strain rats in the first five months of feeding p-DAB but when the liver tumours had developed the death rate at the end of 6th, 7th, 8th and 9th months was 6·5, 9·5, 20·0 and 16·5%. The rate of mortality in the same strain of rats observed by previous workers was from 15 to 70% in the first month of feeding p-DAB without any changes in the livers of the animals. It had also been observed by some workers that the death rate increased to nearly 100 per cent with the progress of neoplastic changes in the liver when the days of feeding the carcinogen increased to 150 days. In the case of Haffkine strain rats on experimental diet with carcinogen no animal died during the course of the first nine months of the experiment.

Incidence of cirrhosis and cancer.— In the *Haffkine strain* of rats fine cirrhotic changes were first seen in the group of animals killed at the end of five months of feeding carcinogen. Of the animals kept on p-DAB for six months, 37% developed liver tumours and at the end of nine months of the experiment 25% of the animals had liver tumours, while the rest of the animals showed degenerative changes of the parenchymatous cells.

The *Wistar strain* rats developed similar cirrhotic changes at the end of three months of the experiment and in four months 25% of the animals developed liver tumours. The percentage of animals developing tumours in the 6th, 7th and 9th month groups were 37, 85 and 62, respectively.

Biochemical changes.—The biochemical changes in the livers of both the strains of animals were studied. The changes in sterol, phospholpids, sulfhydryl compounds and respiratory enzymes studied in the animals kept on experimental diet with and without carcinogen and stock diet at the end of 6 months have been given in the Table below, as an indication of the type of changes studied from the 1st to the 9th month of the experiment.

TABLE

Biochemical changes in the liver of two strains of rats kept on experimental diet with and without carcinogen and on stock diet for six months.

		Cholesterol, mg./g.		Coline, mg./g.		ATP, mg./g.		ATP-as µg/g. unit		Organic phosphorus, mg./g.		Glutathione, mg. %		Cystine, mg. %		Methionine, mg. %		Succinate %		p. $C_6H_4(NH_2)_2$ %	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Stock diet	H	0·26	0·27	3·3	4·1	0·091	0·085	14·0	13·6	7·4	9·7	242·5	223·0	463·6	536·8	599·6	635·6	171·2	149·4	154·7	173·4
	W	0·44	0·43	3·3	3·3	0·053	0·045	12·4	13·6	8·7	8·8	157·2	190·9	787·6	822·8	403·5	453·3	205·5	173·6	210·7	182·2
Experimental diet	H	0·44	0·45	2·9	2·5	0·104	0·094	10·0	11·9	7·9	8·4	198·5	234·9	471·5	563·4	497·6	577·4	146·7	149·6	163·2	153·5
Without carcinogen ..	W	0·54	0·49	2·7	2·8	0·055	0·041	11·5	11·1	11·1	10·4	162·6	205·2	539·8	762·0	255·8	371·7	152·5	174·5	172·9	180·5
Experimental diet	H	0·56	0·73	2·0	2·3	0·095	0·091	7·8	8·9	13·4	14·6	237·8	221·9	476·9	482·4	484·9	487·7	7·7	5·8	10·1	12·7
With carcinogen	W	0·82	1·43	2·0	2·0	0·084	0·072	6·8	6·8	7·8	9·4	200·8	203·2	732·3	708·4	482·4	488·4	10·6	0·5	12·6	—2·8

H—Haffkine rats. W—Wistar rats. M—Male. F—Female.

This experiment has shown that a stimulus for malignant cellular proliferation in the liver of the animals by carcinogen sets in between 3 and 6 months of butter-yellow administration. A weekly study of the animals within this period might show in detail the different degenerative changes taking place in liver cells. It is, therefore, intended to study further the biochemical changes in the livers of the animals between three and six months of feeding the carcinogen.

Investigations on cancer subjects : radiation reaction and the effect of vitamin B on oral cancer patients.— A preliminary clinical and biochemical work was carried out on the oral cancer patients undergoing radiation. The vitamin B levels of these patients were studied before *x*-ray treatment. Owing to the breakdown of *x*-ray apparatus in the course of investigation it was not possible to complete this study.

2. Enquiry on Naga sore under Dr. S. R. Pandit at the Pasteur Institute, Shillong.

The work of the enquiry has been carried out along the lines indicated in the proposals submitted.

During the period under report ten patients with Naga sore were admitted to the Clinical Research Hospital attached to the Institute for investigation. Seven of these patients had active sores, while in the remaining three the sores were of a chronic nature. In all of them the ulcers were distributed over the lower extremities below the knee. Four cases had a single ulcer and the remaining six had multiple ulcers ranging from two to five in number. In six cases there was a history of previous trauma at the site of the ulcers. Of the remaining cases, in one it had appeared spontaneously as a bleb after initial itching, in two the ulcers had arisen from lesions due to scabies, and in the last case a leach-bite was the precursor of the ulcer. There was evidence of malaria in four patients (positive blood finding and/or enlarged spleen). Seven had helminthic infections, one had ankylostome and the rest roundworm infections. A fair number of *E. histolytica* were found in one case. In regard to Wassermann reaction one gave a strong positive while another gave a doubtful reaction. There was no clinical evidence of malnutrition in any of the patients.

Microscopy of smears.— Dark-ground examination and smear examination of discharge were carried out from day to day in each of the cases. The *fusiform* organism was constantly present in each of the ulcers and there was a definite correlation between their intensity and the state of the ulcer; the more acute the ulcer the larger the number of organisms, few of them being seen when healing had proceeded far enough. Interference with healing increased their number. This corroborated the earlier findings.

In none of the cases was a *spirochæte* seen at any stage. It is, therefore, very doubtful whether this organism has any ætiological significance.

Other organisms present in ulcers were *cocci*, which were seen in every case, and in a few of the cases *Proteus vulgaris* and *Ps. pyocyaneus.*

Cultural studies.— The constant association of *fusiformis* with the active ulcer being very significant, a great deal of time was devoted to evolving a suitable selective medium for its easy isolation, but this has not yet been achieved. Only eight isolations have been made from scores of attempts. In the best of

media so far tried, the organism in primary isolation either does not grow at all, or an isolated tiny colony might be found amidst colonies of extraneous organisms, and this even from a discharge which on microscopic examination teems in *fusiform* bacilli with hardly any other organism.

In their study of *fusiform* bacilli, Slanetz and Rettger have shown that potato extract possesses growth-stimulating property. While their medium, with gentian violet, 1 in 10,000, to inhibit other organisms, was found to be excellent in primary cultures of *fusiformis* from teeth and gums, cent per cent isolations being effected in eight healthy individuals, it was not found to favour the growth of *fusiformis* from Naga sore. The elimination of gentian violet and the enrichment with blood 20% was of no avail. During the course of this work it was found that gentian violet was inhibitory to the *fusiform* strains isolated from Naga sore even in a concentration of 1 in 40,000 when introduced in 20% blood agar, the medium used for routine subcultures.

X and V factors had no stimulating effect on the growth of the organism and were not useful in primary isolation. Neither yeast extract nor heated blood gave good results. In fact the growth on " chocolate " agar was, in comparison with blood agar, definitely poorer. Where primary isolations were effected, the phenomenon of " satellitism " was never observed.

Autoclaved pus and L_3 candle filtrate of pus, when smeared on media, were not effective in aiding isolation. Pus-blood-agar recommended by Panja for isolating the organism, did not yield favourable results. Candle filtrates of broth cultures of concomitant organisms isolated from Naga sores, when incorporated in media, were not found to aid primary isolation of the *fusiform* organism.

Hadley Wright's broth with reduced peptone was used for preparing 20% blood agar. Using this medium, two isolations were made from Naga sore but at the present moment it is not possible to state whether this medium is superior to blood agar prepared from trypsin broth, which has been our standard medium.

In isolating the *fusiform* organism from the mouth little difficulty was encountered in primary isolations on Slanetz and Rettger's medium, but subcultivation even on the same or other media was very difficult. With a view to counteract this tendency, broth, saliva (filtered), thick suspensions of *fusiformis* killed at 56° C., for 30 minutes filtrates of suspensions of *fusiformis* frozen and thawed repeatedly, were used, but with little effect. Further work is in progress.

Morphology, etc.—The organism is a strict anærobe and its growth appears to be favoured by the presence of 5% CO_2 in an atmosphere of hydrogen. It is non-sporing and non-motile. Although in smears from pus the bacilli are Gram-positive, after prolonged treatment with alcohol they become Gram-negative. In cultures, however, they were invariably Gram-negative. Their *fusiform* shape is lost in culture but the beaded appearance is seen with Leishman or prolonged staining with Giemsa. They are arranged singly or in pairs end to end. In a few strains the organism grows out into long filaments. The optimum temperature for growth is 37° C. and it fails to grow at 22° C.

In subcultures two types of colonies are observed but the types never breed true. After many subcultures no transformation into spirochætal forms was ever seen and these observations do not lend support to the findings of Tunnicliff who claimed that the *spirochæte* and the *fusiformis* were different phases of the same organism.

The organism ferments none of the sugars used, viz. glucose, lactose, mannite, maltose, saccharose and dulcite. The addition of serum is essential in sugar media. There is indole production but no liquefaction of gelatin.

Agglutination tests were very unsatisfactory as the suspensions agglutinate spontaneously in normal saline. To overcome this low concentrations of saline were used. Even then, this tendency to settle out could not be avoided.

Reproduction of the sore.— Direct inoculation of pus from a sore by scarification or by intradermal injection, reproduces the sore in the human being, but not in laboratory animals—the monkey, rabbit, guinea-pig, white mouse, white rat are completely refractory. Even in patients of Naga sore with an active or healed ulcer, a fresh ulcer could be reproduced by this means, thus showing that immunity conferred by a previous ulcer is not of a solid nature. Out of seven cases, the sore was reproduced in six.

Thick suspensions of a freshly isolated culture of *fusiformis*, injected intradermally into five human volunteers failed to produce the sore. In four of them, there was pain and moderate induration at the site of inoculation during the first 48 hours, but it subsided subsequently. In the fifth a purulent bleb resulted. The aspirated fluid contained no *fusiform* bacilli and healing was rapid. In a patient with healing ulcer with no *fusiform* bacilli on two or three successive days, a culture of *fusiformis* was rubbed into the ulcer but the ulcer did not flare up and no impediment to healing was observed. This experiment repeated in laboratory animals, has also failed.

On the assumption that the sore might be caused by a virus alone or in association with the *fusiform* bacillus, suspensions of pus were filtered through L_3 porcelain candles and the filtrates alone and a mixture of the culture and the filtrate were inoculated separately in four human volunteers by the intradermal route. The filtrates failed to produce any reaction whatsoever and no ulcer could be produced with the combination in any of the volunteers.

As *staphylococci* were found in each and every sore, it was considered possible that these organisms first produced necrosis thereby favouring anærobic surroundings in which *fusiforms* could develop. Experiments were done in three volunteers in whom mixtures of the suspensions of the toxigenic *staphylococcus* strain Wood 86 and *fusiformis* were inoculated intracutaneously. Although there was pain and marked induration at the sites of inoculation no ulcer could be initiated.

The selective distribution of the ulcers on the lower extremities, affecting persons who walk barefooted, seems to support the contention that soil may contain an accessory factor which helps infection. As a kataphylactic agent, such as ionizable calcium salt, has been shown to have the same action as soil in experimental work on tetanus, calcium-chloride solution in varying strengths from 60 mg. to 100 mg. per c.c. was injected along with *fusiformis* cultures in four volunteers. Neither the simultaneous injection of the two nor the injection of the culture over the area previously infiltrated with calcium chloride produced a lesion.

Miscellaneous.—During the year *fusiform* bacillus from the mouth was cultivated from each of several healthy individuals who never had the sore. The morphology of the organisms in stained smears, as well as that of the colonies in cultures, is quite different from that of the *fusiform* organism isolated from Naga sore. This observation does not lend support to the hypothesis of Clements that Naga sore results from infection of cuts and abrasions by saliva containing *fusiformis*.

In view of the fact that penicillin has been very favourably reported upon in the treatment of Naga sore, the bacteriostatic action of penicillin on the strains isolated was tried. They were all sensitive to penicillin.

An opportunity was efforted for the study of the histopathology of Naga sore at different stages of the disease from the blister stage to the chronic ulcer. Tissues from four cases at different stages were kindly provided by Dr. Kempton P. Hare of Upper Assam. Summarizing the results of the findings, in a well-formed ulcer three well-differentiated zones can be seen: (1) a zone on the surface in which fibrin, red cells and polymorphonuclear leucocytes, many of which are disintegrating, are seen; (2) a narrower, wavy, middle zone consisting of *fusiform* bacilli with a palisade arrangement, the bacilli being arranged with their long axis perpendicular to the surface of the ulcer. Below this is (3) a deep zone of œdematous granulation tissue, with newly formed capillaries which tend to run transversely towards the surface. They are very congested, dilated and surrounded by leucocytes, and as they approach the middle zone intravascular clotting is seen in many of them.

Although *fusiform* bacilli are concentrated in the middle zone, they are also met with in clusters in the superficial zone, but they hardly penetrate zone 3. These bacilli were also seen in all the sections, including the earliest stage of the sore—the blister, where they are scattered in the blister fluid along with red cells, cells of the epidermis, polymorphs in various stages of disintegration and a large number of eosinophiles in a loose mesh of fibrin. A noteworthy feature in all the sections is the preponderance of eosinophiles in the cellular exudate.

3. Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

I. During the first three months 47 cases of the fungus involvement of the nails and the surrounding tissues were studied as a continuation of the previous year's study.

Summary of the study :—

Total number of cases	..	256
Positive findings	..	212
Negative	..	44

Positive findings 212—

Ringworm fungus	..	190
Monilia	..	17
Actinomyces keratolytica	..	5

Negative findings 44—psoriasis 13; syphilis 2; tuberculosis 1; bacterial infection 15; allergy 8 and undiagnosed 5.

Divided into two main groups the results are :—

GROUP I: Fungus infection of the nails only—

Number of cases—209

Positive findings	..	190
Negative	..	19

Species isolated—

Epidermophyton rubrum	..	100
E. floccosum	..	63
Trichophyton violaceum	..	1

T. gypseum	..	7
Could not be isolated	..	19

GROUP II : Fungus infection of the tissue surrounding the nails—perionychia.

Number of cases—47

Positive	..	22
Negative	..	25

Positive findings—22

Monilia infection	..	17
Actinomyces keratolytica	..	5

The point of interest and significance is the preponderance of the incidence of *E. rubrum* (*Trichophyton purpureum* of the American workers) over *E. floccosum*—quite contrary to the opinion held so long.

II. *Study of the trichophyton infection of the skin—isolation and identification of the species.*— Altogether 400 cases have been studied and some more cases are still under study. Of these 400 cases the causative organisms, the fungi, were isolated in 359 cases, and in the remaining 41 cases both the microscopic examination and culture were negative.

Method of study.— In each case microscopic findings were confirmed by cultures and the organisms isolated in positive cases were studied for identification of the species.

(1) *Morphology.*— The morphology of the isolated organism and their organs of reproduction were studied from day-to-day growth in welled-slide preparation of the micro-culture in various media. Of the various other media tried for micro-culture preparation wheat starch water was found to give more satisfactory result in the study of the end-organs of the fungus of this group.

(2) *Cultural characters.*— The primary culture was mostly on Sabouraud's proof medium and also on Sabouraud's medium to which gentian violet was added in accordance with the previous work published from this enquiry.

The subcultures were studied in various laboratory media. The various natural media like wheat, barley, oats, etc., were extensively used to maintain the specific characters of the species and to prevent the degenerative changes (pleomorphism) which is common in fungi of this group. The results of study in each case were finalized after six weeks' observation of the growth of the organism.

Briefly stated the results of the study were as follows :—

Number of cases studied—400

Positive finding	..	359
Negative	..	41

Species identified—

Epidermophyton rubrum (*Trichophyton purpureum* of the American workers)		176
E. floccosum	..	166
T. gypseum	..	9
T. violaceum	..	8

In four cases double infection of *E. floccosum* and *E. rubrum* were found.

In cases of interdigital affections of hands and feet care was taken not to

miss the organism and repeated examinations were made whenever necessary.

Interdigital infection—

Number of cases studied—101

Epidermophyton rubrum (*T. purpureum* of the American workers)	56
E. floccosum ..	41
T. violaceum ..	2
T. gypseum ..	2

Conclusion.— It is a point of great interest and significance to note that actually the incidence of *E. rubrum* (*T. purpureum* of the American workers) is greater than *E. floccosum* which is said to be the commoner ringworm fungus infecting the skin. The exact systematic position of *T. purpureum* (*E. rubrum*) is still under discussion. If this species is placed in the genus *Epidermophyton* as it should be then the incidence of *Trichopytosis* of the skin comes to less than 5 per cent and the only two species infecting the skin so far obtained are *T. gypseum* and *T. violaceum* which have already been reported and published.

4. Enquiry into infections of the feet under R.B. Dr. M. G. Kini at the Stanley Hospital, Madras.

This note is in continuation of the one published in the *Report* of the Scientific Advisory Board of the Indian Research Fund Association covering the work done during the year 1946.

A. Sectional Anatomy.

In continuation of the study of cross section anatomy of the foot, two saggital sections of the foot were taken and studied. One of the sections was taken passing through the median line of the foot, i.e. through the axis line of the second metatarsal bone and its phalanges. The other section was taken passing through the interdigital cleft between 3rd and 4th toes and the region between the 3rd and 4th metatarsals. The following points of interest were noted :—

(1) The section of the vein forming the dorsal venous arch was noted to be in the dorsal subcutaneous space. In either section the areolar tissue around the vein held the skin and subcutaneous tissues above and the deep fascia below together. Therefore, it could be appreciated that the dorsal subcutaneous space through which the dorsal venouch arch traverses is demarcated into separate portions, one proximal and the other distal to the dorsal venous arch. The areolar tissue around the vein, however, was not strong.

(2) The web space and its relation to the plantar interdigital subcutaneous space and the dorsal and digital subcutaneous spaces were well brought out in the second section. The lumbrical tendon with its sheath crossed through the proximal part of the web space in the dorsal aspect before it joined the extensor expansion and gained insertion into the base of the proximal phalanx.

A similar study of the sectional anatomy of the foot with reference to the fascial spaces and tendon sheaths is not available in the literature.

B. Injection Experiments.

A mixture of plaster of paris in glycerine and water was injected into the fascial spaces. Radiograms were taken after injection and the limbs dissected and the spread studied.

1. *Dorsal subcutaneous space.*— The experiment noted in the previous report was repeated on another four limbs. The findings already noted were verified to be correct and the following additional points were noted :—

(i) When the plaster mixture was injected into the dorsal subcutaneous space from any point in it the material always showed a tendency to fill up the space proximal to the point of entrance of the needle into the space and not the rest of the part distal to it.

(ii) When injected under pressure the mass broke through the areolar tissue around the dorsal venous arch at more than one point and spread proximal to it.

(iii) The space is lax and affords to puff up considerably, the skin and integument being loosely bound down to the deep fascia by areolar tissue. This suggests that if infection were severe, swelling would be intense and the skin stretching under great pressure may be impoverished of the blood supply resulting in extensive destruction and necrosis.

(iv) The small sephanous vein passes behind the lateral malleolus and the space below and behind it would well up with the material extending from the dorsum of the foot. The great sephanous vein passes in front of the medical malleolus and the material would not extend below and behind the medial malleolus. However, when more material was injected, it spread up in front of the ankle to the subcutaneous space in the lower third of the leg, where it is continuous all round the leg. The subcutaneous space behind the medial malleolus being continuous with the subcutaneous space of the leg proximal to it, the material filled up the space behind the medial malleolus from above.

These details are not given in the literature.

2. *Dorsal subaponeurotic space.*— It was observed in the previous report that " the deep or aponeurotic layer of fascia permits tunnelling by the extensor tendons as they course down the foot. Fine areolar tissue binds down the aponeurotic layer of fascia to the third deeper layer of the fascia along the length of the metatarsals and on either side of the extensor tendons. Thus, the sub-aponeurotic space is subdivided into a number of longitudinal compartments". In the present series of experiments plaster mixture was injected into the dorsal sub-aponeurotic space around the tendon slips of the extensors digitorum. It was found to spread up and down these tendon slips and the following points noted :—

(i) The sub-aponeurotic space is divided into many anterio-posterior compartments.

(ii) The sub-aponeurotic space around the distal part of the extensor digitorum longus tendon and its synovial sheath, branches out along with each digital slip of the tendon, whereby these spaces along the length of each tendon slip communicate with one another only in their proximal extent.

(iii) The spaces along the tendon slips of the extensor digitorum brevis to the second, third and fourth toes communicate with the space deep to that part of the extensor digitorum brevis muscle from which they arise and hence with one another, proximally.

(iv) In all the three limbs injected and dissected, the part of the extensor digitorum brevis muscle which contributed towards the formation of the tendon slip to the hallux was separate from the rest of the extensor digitorum brevis muscle. The space along the tendon slip from the extensor digitorum brevis to the hallux is continuous with the space deep to that part of the extensor digitorum brevis from which the tendon slip arises, and is separate from the space behind the rest of the extensor digitorum brevis muscle and the spaces round the tendons arising from it.

(v) Each of the spaces between 1 and 2, 2 and 3 , 3 and 4, and 4 and 5 metatarsal bones was separate from the others and extended from the head to the base of the bones on either side of it. The spaces along the digitorum tendon slips (long and short) pass obliquely, superficial to the subaponeurotic space between the metatarsal bones.

(vi) Each compartment is a fairly compact one and it is suggestive that infection in one may not easily spread into the others.

(vii) In one instance when material was injected under pressure into the space along the tendon slip of the longus digitorum to the second toe it was found to spread up to the distal part of the tendon and from there distally along the tendon slips going to the other toes. When some more was pushed in, it was found to have burst into the distal part of the tendon sheath of the extensor digitorum longus and spread up proximally. The material again ruptured the tendon sheath in its proximal extent and spread up between the muscle and its mycelial sheath in the anterior compartment.

None of these observations are found in the literature.

3. *Median plantar spaces—M-I.*—The plaster mixture on injection filled up the space anterio-posteriorly part by part. The space was found to be divided into four compartments which communicated with each other proximally. The material from M-I spread round the lateral borders of the flexor digitorum brevis and between groups of muscle bundles deep into M-II.

M-II.— Material spread from M-II to M-III in all cases (100%) round the lateral borders of the flexor accessorius and the crossing of the flexor tendons. In 50% of the cases it passed through the medial plantar septum and spread proximally along the tendon of flexor digitorum longus deep into the medial leg space. In one of the cases it spread along the tendon of flexor hallucis longus distally in a subcutaneous space lying between the tendon and the septum. In another case it spread along the superficial branch of the lateral plantar nerve piercing the lateral plantar septum into a subcutaneous area lying between the septum and the flexor digiti quinti brevis. In another case it spread into the lateral plantar space along a branch of the lateral plantar artery piercing through the septum.

M-III.— Material spread into M-IV in all cases (100%). It spread into M-II in 50% of the cases. Distally it was found to spread deep to the lumbricals and then around them towards the interdigital subcutaneous spaces.

M-IV.— Material spread into M-III in two cases. In one it spread through the first interosseous space and between the muscle fibres of the first dorsal interosseus into the dorsum of the foot. In another it spread laterally deep to the transverse head of the adductor hallucis.

4. *Lumbrical spaces.*— Attempts at injecting lipoidal and glycerine-plaster of paris mixture into those spaces in dissected specimens were futile owing to certain practical difficulties. Further measures to circumvent these will be taken in further work.

5. *Medial plantar space.*— In all cases material became subcutaneous all along the medial border of the foot. In one case the spread was noted along the medial plantar vessel and nerve proximally into the medial leg space and distally to M-III.

6. *Lateral plantar space.*— The material bulged out subcutaneously along the lateral border of the foot.

7. *Medial plantar space-II.*— The spread was subcutaneous along the medial border and into M-IV adjacent to it, wherefrom spread took place to the other areas.

Injection experiments with regard to these spaces and others is being continued.

C. Interdigital Subcutaneous Spaces.

1. There are four subcutaneous spaces in connection with each interdigital region in the sole of the foot. They are roughly triangular in shape with the apex pointing proximally. The base is formed by the superficial transverse planter ligament and the sides by the digitations of the central part of the plantar aponeurosis. Superficially they are bounded by the skin. The space is filled with lobules of subcutaneous fat. The common digital nerves and vessels and the lumbricals with their fascial sheath pass through the depth of the space. The space extends into the web distally deep to the superficial transverse plantar ligament, and hence through it communicates with the dorsal subcutaneous space.

Infection from the interdigital subcutaneous space may spread distally into the corresponding web space and the dorsal subcutaneous space by contiguity and proximally to M-I along the common digital nerves.

Plaster mixture was injected into these spaces and the spread noted in each case. The material spread in all cases (100%) into the corresponding web space. In some cases it spread from there into the neighbouring web space and its corresponding interdigital subcutaneous space. Radiograms to illustrate were obtained.

Clinical study.— A number of cases with infection and abscess formation in the interdigital subcutaneous spaces were studied. The abscess always tried to localize itself to the space. In all cases there was lymphatic œdema of the dorsum of the foot and in the corresponding web space. In about 30% of the cases pus spread into the corresponding web space by contiguity. In three cases pus localized in the dorsal subcutaneous space of the foot, the corresponding web space having escaped and the spread taking place via lymphatics (these cases came with long duration). In all the three cases the localization was distal to the dorsal venous arch.

A detailed study of the surgical anatomy, ætiology, pathology and treatment of pyogenic infection in the interdigital subcutaneous and web spaces is being continued.

2. *Subcutaneous space in the heel.*—Certain amount of study with regard to the subcutaneous space in the heel was made in the preliminary work done in the " Infection of the foot " in 1944-45 and a note published in the *Indian Journal of Survey* (8, Nos. 1, 2 and 3, 1946) as a part of the assay on the " Infections of the foot ". Further study of surgical anatomy, ætiology, pathology and treatment of pyogenic infections of the heel space is now being made.

3. *Tropical ulcers.*—34 cases of tropical ulcers have been studied with regard to the culturing of the *Bacillus fusiformis* and the role of penicillin in the treatment of these ulcers. In 5 out of 12 cases positive cultures of *B. fusiformis* were obtained. Subcultures could not be grown from them. Twenty-eight of the cases were dressed with penicillin in a dilution of 1,000 units of penicillin in 1 c.c. of sterile, pyrogen-free distilled water. Fourteen of these cases were discharged cured. Six others stayed away from attending the hospital when the pain had subsided, and wound was free from discharge and epithelializing rapidly. Attempts at tracing them proved futile. The rest of the 8 cases attended the hospital for a few days and stayed out before they derived any benefit out of the treatment given. Ulcers with duration of less than month and with a diameter of not more than an inch healed within 10 days, whereas those with longer duration and more than an inch in diameter in size took nearly four weeks to heal. The presence of *B. proteus* and *pyocyaneus* in the ulcers slowed down the pace of healing, one of the cases taking more than six weeks to heal. The average quantity of penicillin required to treat a case of tropical ulcer was found to be 8,000 units, the maximum being 14,000 units. They counteract the action of *B. proteus* and *pyocyaneus*, the use of *Streptomycin* is comtemplated.

An intensive clinical study in the light of the experimental study is being done to evaluate the experimental observations.

5. Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

I. Experimental Hepatic Cirrhosis.

A summary of the previous experimental work on the effect of continued feeding of diets containing 18, 10, 8 and 5 per cent portein (casein) with and without the supplements of vitamins of B_2 complex on the histological and biochemical changes in the liver of rats was described in the last *Report* (1946).

It was realized from previous results that it was the daily intake of casein and not its percentage in the diet that is necessary to restrict. Accordingly, four groups of rats were given synthetic diets in such quantities that rats from the first group received a maximum of 1·2 g. of casein per rat per day, while those in the second, third and fourth groups received 0·6 g., 0·4 g. and 0·2 g. of casein respectively per rat per day. Each rat was given in addition 0·5 g. of yeast per day. An outline of the experimental procedure was described in the last *Report*. A summary of the gross chemical changes and histological appearances of the liver are recorded below :—

A. *Effect of restricted intake of casein in an otherwise adequate diet on the histological and biochemical changes in the liver of rats.*

Histological findings.—On microscopical examination, the liver in animals which received 1·2 g. and 0·6 g. of protein per rat per day showed no pathological changes. Animals which received 0·4 g. and 0·2 g. of protein per rat per day, on the other hand, showed slight fatty change of the hepatic parenchyma around the central veins. In marked contrast to the animals in the low protein groups which were deprived of the factors of the vitamin B_2 complex, none of the animals in this group, however, showed marked degenerative changes in the hepatic parenchyma around the central veins, accumulation of 'ceroid' pigment or diffuse hepatic fibrosis.

Gross chemical changes.—The liver of every individual rat was analysed separately for water, total fat, protein and ash content.

Water.—The average amount of water in liver calculated as g./100 g. body-weight, was practically the same in the first three groups, but its amount was slightly increased in the fourth group of rats.

Fat.—The amount of liver fat showed a gradual increase in different groups as the daily intake of casein was progressively reduced.

Protein.—The amount of liver protein diminished with the progressive decrease in the intake of casein in different groups of rats except in the fourth group.

Ash.—The ash content varied in the same way as the liver protein.

Liver-weight.—The liver-weight as g./100 g. body-weight was definitely increased in the fourth group as compared with other groups.

Choline content of the liver.—The choline content of the liver as mg. per g. of fresh liver-weight was found to be definitely higher with the increase in the intake of casein from 0·2 g. to 1·2 g. per rat per day. The average choline content of the liver of rats receiving 1·2 g. of casein per day was found to be 2·14 mg. per g. of fresh liver, whereas those in the other three groups, it was respectively 1·89 mg., 1·43 mg. and 1·55 mg. per g. of fresh liver-weight. These results are important in view of the relation of choline to fatty and other histological changes in the liver and requires to be repeated for confirmation.

Total plasma-protein concentration.—The daily intake of casein was found to have a profound influence on the total protein concentration of the blood of rats. The average total blood protein was 6·83 g., 6·11 g., 5·52 g. and 4·81 g. per 100 c.c. plasma in the four different groups receiving 1·2 g., 0·6 g., 0·4 g. and 0·2 g. of casein respectively per rat per day. This shows that in the first and second group of rats, the total plasma-protein was normal and their livers were also normal. In the third and fourth group of rats, on the other hand, the total plasma-protein was below normal, the fourth group showing a definite hypoproteinæmia. Slight pathological and chemical changes in the liver with hypoproteinæmia are indicated from both microscopic and chemical examinations of livers but it appears that low intake of protein alone produces a more clearly demonstrable effect on blood protein concentration than on liver structure.

B. *Investigation of the lipotropic factors specially choline in the production of experimental cirrhosis.*

Two dissimilar diets one containing high fat and low protein and the other containing high carbohydrate with low protein were used for production of liver cirrhosis in rats.

(a) *Effect of high-fat—low-protein diet.*—High-fat diet was composed of casein—6%, dalda—50%, cod-liver oil—1%, starch—20%, sucrose—20% and salt mixture—3%. The diet was supplemented with adequate amount of vitamins including thiamine hydrochloride, pyridoxine, riboflavin and calcium pantothenate. Young rats kept on this diet showed a considerable decline in body-weight. A number of them developed œdema and ascitis. A few of the animals died. Others were killed during periods varying from 33 to 110 days. The livers were enlarged in all cases and the average liver-weight per 100 g. of body-weight was 6·33 g. (In normal rats the liver-weight varies between 4 g. and 4·8 g. per 100 g. body-weight). The increase in the weight of the livers was due to the deposition of fat to the extent of 25·4% of fresh liver. A preliminary microscopic examinations of the sections of liver also suggests fatty infiltration with diffuse hepatic fibrosis. The detail microscopic examinations are in progress. The average choline content of liver was 1·06 mg. per g. of fresh liver-weight. (In normal rats, liver contains 2 mg. to 3 mg. of choline per g. of fresh liver). The average value of the total plasma-protein in the blood of this group of rats was 3·77 g. per 100 c.c. plasma which is considerably below normal.

(b) *Effect of high-carbohydrate—low-protein diet.*—The high-carbohydrate diet was composed of casein—6%, starch—42·5%, sucrose—42·5%, groundnut oil—5%, cod-liver oil—1% and salt mixture—3%. The diet was supplemented with synthetic vitamins. Animals from this group showed a decline in body-weight in the initial period and subsequently maintained a constant body-weight and lived much longer than those on the high-fat diet. There was no œdema, ascitis and death in this group. All the rats were killed. The average liver-weight in this group of animals 4·70 g. per 100 g. body-weight. The percentage of fat in the liver was 19·8 and was less than that of high-fat group. The choline content of the liver was practically same as on high-fat diet and was found to be 1·02 mg. per g. of fresh liver. The total plasma-protein concentration was higher in this group of animals than those on high-fat diet and the average value was 5·22 g. as compared to 3·77 g. per 100 c.c. plasma on the high-fat diet. The results of albumin and globulin fractionation of the blood-protein were as follows: Total protein—5·38 g., albumin—2·97 g. and globulin—2·41 g. It is seen that low total protein was due to the decrease in the albumin fraction, globulin showing the normal value. The microscopic examinations of livers are in progress.

(c) *Influence of choline on the causation of hepatic cirrhosis on a high-fat—low-protein diet.*—Choline in doses of 4 mg. per rat per day was given along with high-fat diet. The supplements of choline had no effect on the food consumption of the rats nor did it prolong the survival period of rats. The liver-weight of this group of animals was 5·62 g. per 100 g. body-weight. The effect of 4 mg. choline of supplements in removing fat from liver was very significant. Liver fat was reduced to 9% as compared to 25·4% on fresh liver on high-fat diet alone. The addition of choline, however, did not produce any change in the choline concentration of the liver. The choline content of the liver was 1·18 mg. per g. of fresh liver-weight. The average value of total plasma-protein was 4·93 per 100 c.c. plasma and was higher than those fed on high-fat diet alone. The microscopic examinations of the livers are in progress.

(d) *Influence of methionine on hepatic cirrhosis on a high-fat—low-protein diet.*—Methionine in doses of 20 mg. per rat per day was given along with high-fat diet. The supplements of methionine had a favourable effect on the food

consumption of the rats specially during the early part of the experiment and the rats lived much longer than those on high-fat diet with choline supplements or on high-fat diet alone. A few of the animals died and others were killed during the periods varying between 99 and 162 days. Histological and biochemical examinations of the livers are in progress.

(e) *Microscopic examination of the sections of livers.*—The detail study of the sections of liver from different groups including those receiving choline and methionine is being carried out by Dr. M. V. Radhakrishna Rao at the University College Hospital, London, in collaboration with Prof. H. P. Himsworth.

II. Clinical Investigation on Cirrhosis of Liver.

A clinical study of cases of cirrhosis of liver is being carried out with Dr. N. J. Modi, M.B.,B.S., M.R.C.P.(Lond.), in his medical unit at the Goculdas Tejpal Hospital. It was planned to study these cases from the point of view of ætiology, routine investigations including total plasma-proteins and blood-choline and to note the effect of treatment by giving high-protein diet, yeast tablets, crude liver and vitamin B-complex injections on alternate days.

Ten cases of cirrhosis of liver have been studied. Out of these six cases have been discharged cured, with no evidence of fluid in the abdomen. Two advanced cases died, one case went home against medical advice after one month's stay in the hospital without showing any improvement and the other went away after 8 days' stay against all medical advice.

Most cases showed low total plasma-proteins, particularly albumin which increased considerably as they improved.

The blood-choline estimations show a wide range of figures, the lowest was 7·6 mg. per 100 c.c. and the maximum was 28·32 mg. To get an idea of the normal figure, blood-choline of healthy members of the house staff and medical students has also been estimated. The results are significant.

6. Enquiry on cirrhosis in animals and its bearing on the ætiology of infantile biliary cirrhosis under Dr. T. Bhaskara Menon at the Andhra Medical College, Vizagapatam.

Five sets of experiments have been carried out with sucklings and young animals (puppies and rabbits). More or less clear-cut results have been obtained. A sixth set of experiments is being carried out.

The first set of experiments was with young puppies fed with pure castor oil in large doses (3 c.c. per kg. body-weight). None of the animals survived more than 7 days. This was regarded as due to inanition from chronic diarrhœa; no significant changes in the liver were noticed. In the second set of experiments, animals were fed during the entire suckling stage and early weanling stage with smaller doses of castor oil varying from 0·3 c.c. per kg. body-weight, gradually increased to 0·6 c.c. per kg. body-weight. The period of experiments ranged from 50 to 100 days. In the later stages slight degrees of fatty degeneration were noticed. Cirrhosis was not met with. In the third set of experiments young rabbits were fed with pure olive oil (0·5 c.c. per kg. body-weight) from periods ranging from 50 to 140 days. There were no significant results except slight fat-loading in the liver. In the fourth set of experiments

suckling puppies were fed with crude castor oil (0·4 c.c. to 1 c.c. per kg. body-weight). Except for slight fat-loading no cirrhotic changes were observed. The sample of this castor oil was carefully analysed for the presence of 'ricin' chemically and by the agglutination test. No 'ricin' could be detected. This was confirmed by the Professor of Chemistry of the Andhra University. In the fifth set of experiments young puppies were fed with clove oil (0·01 c.c. per kg. body-weight). On histological examination of livers fat-loading was observed in the peripheral zone. No cirrhotic changes supervened.

It was more or less clear from these five sets of experiments that increase of fat in the diet was not of sufficient importance as a factor in inducing cirrhosis in sucklings and weanlings in puppies and rabbits.

In a sixth set of experiments now being carried out with pregnant bitches and lactating bitches a standard choline-deficient diet is used to determine the effect of choline deficiency in the diet on the livers of sucklings. These experiments are now in progress. How far choline deficiency in the mother's milk will induce liver lesions in the young is a problem that is of importance in infantile biliary cirrhosis.

7. Enquiry on infantile cirrhosis under Dr. P. Kutumbiah at the Stanley Medical College, Madras.

Clinical.

A careful note of the dietetic, family history, and clinical and serological examinations was undertaken. The condition of the liver and spleen was particularly noted.

Two cases were treated with choline citrate. One was an advanced case, in the third stage, with jaundice and ascitis, and the other was an early case in the pre-ecteric phase. The advanced case died within a week of the commencement of the treatment. The other case, i.e. one in the pre-ecteric stage, has responded well to treatment, the spleen being no longer palpable and the liver, though of the original size, considerably softer than before. This case is being followed.

Biochemical.

Investigations on the dietary deficiency aspect of infantile cirrhosis of the liver were centred on the analyses of mother's milk for the following :—

(1) The sulphur containing amino-acids, methionine and cystine and

(2) The lipotropic factor choline.

Preliminary work was concerned with the investigation of suitable methods for analysis. Values for normal standards were obtained using the following methods :—

(i) Sullivan's colorimetric method was used for cystine.

(ii) Methionine was estimated colorimetrically by the method of McCarthy and Sullivan (*Jour. Biol. Chem.*, **141**) using the sodium nitroprusside reaction.

Choline.—Choline was determined colorimetrically using the photo-electric colorimeter according to the method of Engel (*Jour. Biol. Chem.*, **144**).

It was found that for each analysis at least 12 oz. of milk were required. The usefulness of some of the above chemical methods in view of the difficulties of getting such large samples of mother's milk, is obviously limited.

In the case of choline estimation, a microbiological method has been developed making use of a " Cholinless " strain of the fungus neurospora crassa. (The particular strain was obtained from the United States of America from Dr. Horrowitz). A determination of choline can be made on a 10-c.c. sample of human milk. The original method of Horrowitz and Boadlo (*Jour. Biol. Chem.*, 1943) has been adopted with slight modifications.

The composition of normal human milk and cow's milk were found to be—

	Cow's milk in mg./100 c.c.	Human milk in mg./100 c.c.
Methionine ..	93	30
Cystine ..	28	41
Choline ..	9	5

Three samples of mother's milk in cases where the children had cirrhosis gave the value for choline content as low as 2 mg. per 100 c.c.

Microbiological methods for estimation of the amino-acids, methionine and cystine, using the strain *Lactobacillus arabinosus* (obtained from the United States of America) are being developed. Due to difficulties in obtaining pure amino-acids, essential for this work, progress has been slow.

In connection with the bacteriological work, mention must be made of the co-operation and help rendered by Dr. K. V. Venkataraman and his staff of the King Institute of Preventive Medicine, Guindy, Madras.

The use of these microbiological methods will facilitate the analysis of such small samples of breast milk as are usually available.

The comparative composition of cow's and human milk is significant, i.e. the ration of cystine : methoinine is 1 : 3 in the case of cow's milk, while in human milk it is 4 : 3. Also, there is less choline in human milk than in cow's milk.

8. Clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Profs. V. Subrahmanyan and V. R. Naidu at the Indian Institute of Science, Bangalore and the Sri Krishnarajendra Hospital, Mysore.

Studies on the above enquiry were commenced towards the end of May 1947. In accordance with the suggestions made by the Clinical Research Committee, the investigation was confined to a study of the therapeutic action of choline chloride in the various stages of the disease.

During the above period, 36 cases at different stages of the disease were studied and treated with choline chloride as against a control group of 26 cases treated with pepsin-protcolysed polyglandular extract of the liver, spleen and the pancreas supplemented with choline chloride. Most of the cases were treated as out-patients and were under daily observation of the Medical Assistant who also visited them in their homes whenever necessary. A few beds in one of the wards had been set apart exclusively for cases of infantile hepatic

cirrhosis. A few select cases were hospitalized for short periods for special study as also for extra treatment.

Diagnosis and prognosis.—The provisional classification of the disease into various stages and the related clinical symptomatology have been described in previous reports and also in the original programme submitted to the Indian Research Fund Association last year. The main basis of the classification is the extent of the enlargement and consistency of the liver and the spleen. In the assessment of the exact pathological stage of the disease as well as prognosis under treatment in each case, liver function tests—particularly the thymol turbidity test—and icteric index were used as confirmatory acids to clinical symptoms.

Treatment.—The group treated with choline received pure choline chloride (B.D.H.) in the form of a 25% faintly acid solution in 10% alcohol. The solution was kept faintly acid to prevent hydrolysis and the alcohol served to protect it against bacterial infection. The dosage was 250 mg. of choline chloride administered twice daily before food. The total quantity given to a child per day (500 mg.) constituted 10 to 12 times the maintenance dose of choline chloride (1 mg./100 g. body-weight) judging on the basis of the data for experimental animals, the average body-weight of the children treated being 10 lb. to 12 lb. In order to render the bitter drug as palatable as possible it was sweetened by admixture with sugar or honey prior to administration.

The control group was treated with the pepsin-proteolysed extract of liver, spleen and pancreas employed in our previous studies as supplemented with 140 mg. of choline chloride per child per day. The preparation of the glandular hydrolysate, its composition and dosage are described in the original programme submitted to the I.R.F.A.

In all cases an adequate supply of vitamins was ensured by the administration of yeast or B-complex preparations supplemented with vitamin C and adexolin or multi-vitamin preparations.

Secondary complaints like constipation, temperature, anæmia, etc., and complications like distension, œdema, jaundice, etc., were treated symptomatically. To counteract jaundice in the last stage of the disease dehydro-choline was given orally and parenterally and a 25% solution of glucose containing vitamin C (250 mg.), vitamin B_1 (25 mg.) and nicotinic acid (25 mg. to 30 mg.) and 5 c.c. of 30% hexamin intravenously. There was, however, little benefit from these at this stage of the disease.

The dietary regimen of the children was restricted during treatment to cow's milk without much cream, rice with vegetable soup and butter-milk, fruit juice and barley water. Sugar and glucose were allowed in liberal quantities. Fats and fatty foods were rigorously precluded. The dietary availability of calories was about 1,000 per day per child.

Clinical results.—In addition to the recession of the enlarged liver and spleen and remission of other concomitant symptoms the specific criteria of clinical improvement have been: (i) absence of excess fat in and restoration of the normal colour of the fæces, (ii) absence of amorphous phosphates in the urine, (iii) stimulation of appetite and digestion, (iv) gain in weight and (v) development of physical and mental alacrity. Liver-function tests—particularly the thymol-turbidity test—have been employed to confirm clinical recovery. A case has been considered cured clinically when the liver and spleen have receded to the normal size, the clinical symptoms have been

completely relieved and the general condition has been restored to normal. Tables I and II summarize the clinical results :—

TABLE I.

Treatment with choline chloride only.

Pathological condition.	Total number treated.	Number cured.	Number died.	Number discontinued.	Per cent cured.	Period of treatment for effecting cures.
Stage I	8	8	..	..	100	3-5 weeks
State II A	16	16	..	..	100	6-12 „
State II B	8*	4	1	1	50	16-18 „
Stage III	4	..	4	..	0	..

* Two of these cases are still under treatment. The prognosis in both is, however, unfavourable.

TABLE II.

* *Treatment with polyglandular extract supplemented with choline chloride.*

Pathological condition.	Total number treated.	Number cured.	Number died.	Number discontinued.	Per cent cured.	Period of treatment for effecting cures.
Stage I	9	9	..	..	100	2-4 weeks
Stage II A	24	24	..	..	100	2-6 „
Stage II B	16	9	4	3	56·3	8-10 „
Stage III	18	..	18	..	0	..

* For the sake of proper comparison the results in the present series of cases in this group have been combined with those previously obtained.

The pathological stage referred to in the above column defines the clinical condition of the patient at the commencement of treatment. The period of treatment has been reckoned from the day on which treatment was begun to the day on which there was no palpable enlargement of the liver.

All recovered cases have been treated with the glandular extract supplemented with choline chloride for 8 to 10 weeks after complete recovery in order to prevent possible relapses. This treatement was adopted also in the cases treated with choline chloride with a view to economise on the rather limited stock of choline chloride. A number of cases that have been completely cured are still under periodical observation after cessation of treatment. The following clinical observations are of importance in regard to the therapeutic action of choline chloride and its comparison with that of the glandular hydrolysate. In almost all cases choline chloride relieved constipation presumably by stimulating intestinal peristalsis. In the group treated with choline chloride the change in the pale colour of the stools to the normal, which is indicative of the stimulation of bile secretion, took place in 12 to 14 days as against 5 to 6 days in the control group. Motions were rendered non-greasy and excessive amounts of fatty acids disappeared from the stools in two to three weeks in the choline-

treated cases, whilst this happened in about a week in the other group. Choline chloride did not appear to stimulate appetite until after a long period of treatment, whereas marked stimulation of gastric secretion and appetite has been found to be one of the important therapeutic properties of the glandular extract. General improvement was also slower in the choline-treated group.

Of the cases in the third stage treated with choline chloride only in one case a transitory relief from jaundice was noted. There was, however, a relapse after three days.

Cases which resisted treatment in stage II-B gradually deteriorated developing jaundice ascites and œdema and finally ended in death.

Laboratory Studies.

(i) *Pathological and biochemical findings.*—Routine pathological examination of the blood and excreta was carried out in all cases. In some of the cases in the terminal stage the ascitic fluid was also examined. No pathogenic bacterial organisms were found in the blood, the ascitic fluid or the excreta.

The present studies of the blood picture in the disease confirmed our previous finding that in the early stages (I and II-A) it is one of hypochromic normocytic anæmia, whilst in the later stages the anæmia is of a mixed type—micro- and macro-cytic with a relatively large number of macrocytes. Definite leucocytosis and polygorphonuclear leucopenia were found in all the stages.

The coagulation time, the bleeding time and the thrombocytic count of the blood, as also blood-sugar, blood-calcium and blood-urea were within the normal range. The van den Bergh reaction was negative in all but the third stage where it was positive and delayed.

The ecteric index in the early (I and II-A) stages was 4 mg.%. It was 5 mg. to 10 mg.% in the II-B stage and 20 mg. to 40 mg.% in the III stage. The normal range is 0·5 mg. to 3 mg.%.

Fæcal fat analyses for total fat and fatty acids were carried out in a large number of cases prior to treatment. The fæces were collected over a period of 2 to 3 days and as completely as possible. It was preserved in alcohol and then dried over a water-bath. The method of analysis was essentially the same, as the second soxhlet method described in Harrison's 'Chemical methods in clinical medicine' (1944). Table III embodies some typical results:—

Table III

Fœcal fat analysis.

Case No.	Sex.	Age		Diet.	Total fat per cent.	Degree of splitting fatty acids as % of total fat.
		Yrs.	Ms.			
Pathological stage I						
37	Male	..	10	Cow's milk	36·3	85
16	M	1	2	—do.—	40·0	70
41	Female	1	3	Rice and milk	38·9	72
39	M	..	10	Cow's milk	26·8	90

TABLE III (*concld.*)

Case No.	Sex.	Age		Diet.	Total fat per cent.	Degree of splitting fatty acids as % of total fat.
		Yrs.	Ms.			
Pathological stage II-A.						
12	Male	..	6	Cow's milk	34·6	80
23	M	1	3	—do —	52·0	75
33	F	2	..	Rice and milk	43·8	73
34	M	..	10	Cow's milk	45·6	90
32	F	..	10	Breast milk	40·0	78
25	M	2	6	Rice and milk	39·1	65
59	M	1	10	—do.—	43·3	83
Pathological stage II-B.						
24	Male	5	..	Rice and milk	41·7	66
19	M	1	6	—do.—	46·0	..
11	M	..	11	Cow's milk	48·0	70
31	M	2	6	Rice and milk	50·5	65
38	M	..	7	Cow's milk	48·8	65
Pathological stage HI.						
21	Male	..	10	Cow's and breast milk	66·5	..
27	M	2	6	Rice and milk	55·0	45
35	M	1	6	—do.—	60·0	55

During the period of study some of these children were on a dietary regimen of cow's milk and others on cow's milk *plus* rice. The total ingested dietary fat has been computed to be 15 g. to 20 g. per day.

Judging on the basis of the findings in normal children, the total fat excreted in this disease is distinctly above the normal average (30 to 35% of the dry fæces), whilst the degree of splitting is normal excepting in the terminal phase of the disease. The lower degree of splitting in the last stages may be due to impairment of pancreatic secretion or obstruction of the pancreatic duct, or alteration of the intestinal reaction or other factors. The above results clearly indicate that pancreatic efficiency is normal in this disease and that defective absorption of fatty acids, owing to insufficient bile secretion or other causes, is the main cause of the excessive excretion of fat.

The percentage absorption of dietary fat could not be evaluated in the above cases as many of them were out-patients and hence quantitative collection of fæces could not be ensured. We have now planned to carry out strict fat metabolism studies in a few select hospitalized cases before and after treatment as well as other tests of pancreatic efficiency. Such studies will be carried out with normal children also.

Plasma levels of fatty acids, lipid phosphorus (phospholipid) and cholesterol have been estimated in a few (5) select cases. These investigations are being continued to obtain further data at different stages of the disease and after different types of treatment as well as in normal children. The above study will help to elucidate the mechanism of the therapeutic action of choline and the glandular hydrolysate by themselves and in combination. Any important findings on this aspect will be included in the annual report to be submitted in December 1947.

Liver-function tests.—The thymol-turbidity test was the routine liver-fuction test employed for diagnosis as well as for prognosis. It was carried out according to MacLagan (*Nature*, 1944, **154**, p. 670.) using Burroughs Wellcome Vaccine Standards for the quantitative estimation of the turbidity.

In normal cases the reaction is mostly negative and in some cases very faintly positive the turbidity being less than one unit. In all cases of infantile hepatic cirrhosis the reaction was found to be positive, the turbidity readings being high in the I and II stages (5 to 7 units) and low in the II-B (3 to 4 units) and III (2 to 3 units) stages. We have found that a low turbidity reading (below 4) with persistent or deteriorating clinical symptoms is indicative of unfavourable prognosis. Such cases have invariably ended in death. We find that in assessing hepatic damage in this disease, the test is of both diagnostic and prognostic value in conjunction with clinical symptoms.

The methylene-blue test, consisting in reckoning the number of drops of one per cent methylene-blue solution required to render 5 c.c. of urine to a permanent blue colour, was conducted in a number of cases at various stages of the disease. In the I and II-A stages, the urine required 3 to 4 drops, in the II-B stage 4 to 5 drops and in the III stage 5 to 40 drops depending on the intensity of jaundice. The normal range is 2 to 5 drops. The test was thus found to be of no diagnostic value in the early stages wherein laboratory tests are most essential. It may be employed in the III stage as a measure of the depth of jaundice. We have recently received standard cephalin cholesterol and bromsulphalein which we had ordered for, for liver function tests. We are now carrying out comparative studies on the different liver function tests at various stages of the disease.

Histopathology of the liver biopsies.—Liver biopsies were performed on the two following cases in the advanced stage of the disease by the technique of aspiration biopsy using the Vim-Silverman needle and taking the necessary precautions against possible hæmorrhage and other complications (Sherlock, *Lancet*, 1945, **249**, p. 397.).

1. A Hindu boy-child 1½ years of age, pathological stage III, having jaundice and œdema of the feet. The thymol-turbidity reading was 2 units. The liver was 3 fingers below the costal line and hard in consistency. The spleen was enlarged by 3 fingers-breadth. The biopsy was conducted 48 hours before death. Histological examination of the liver revealed sub-acute necrosis, fatty infiltration and round cell infiltration. Culture of the biopsy material showed no growth of Gram-negative organisms. Subculture revealed the presence of *Staphylococcus albus*, probably of extraneous origin.

2. A Muslim boy-child 1 year and 8 months old, pathological stage III, having jaundice and ascites. The thymol-turbidity reading was 2. The liver was hard and granular and enlarged to the extent of 4½ fingers. The spleen was hard and enlarged by 3 fingers-breadth. The biopsy was conducted a week before death. Histological examination of the liver revealed sub-acute or diffuse necrosis, with fatty degeneration, small cell lymphocytic infiltration and also some attempts at repair as judged by the presence of fibroblasts. Culture of the biopsy specimen on agar and Endo's media did not show any growth after 48 hours.

Discussion.—Further clinical and laboratory studies on the therapeutic action of choline are in progress. The trend of the present results justify certain conclusions indicative of further lines of work.

A comparison of the clinical results obtained with choline chloride and the polyglandular hydrolysate supplemented with choline chloride shows that choline chloride is no more effective in the later stages of the disease than the latter combination although the dosage of choline chloride administered when given alone exceeds 3½ times that given along with the glandular hydrolysate. In regard to rapidity of therapeutic action, as judged by the period of treatment required for effecting clinical cures and also stimulation of appetite and bile secretion, reduction of fæcal fat, etc., the combination is definitely superior to choline chloride alone. This is presumably due to stimulation of gastric and hepatic functions in addition to any other specific action of the glandular hydrolysate.

Previous clinical studies have shown that mere cholagogues and vitamins of the B-complex, although they produce general improvement, fail to arrest the disease even in the early stages. We have also found that casein hydrolysate when administered alone is effective only in the early stages of the disease. Both in regard to the period of treatment for effecting clinical cures and the efficacy in the more advanced stage of the disease, we have had the best clinical responses (83 per cent cured in the II-B stage) to treatment with a combination of the glandular hydrolysate choline chloride and casein hydrolysate. The present clinical results taken along with the former clearly indicate the need for the provision of certain active principles in addition to protein and lipotropic factors (choline) in the advanced stages of the disease. The superior efficacy of the glandular hydrolysate in conjunction with lipotropic and protein factors must be attributed to active principles which function directly or in association with these factors. Such active principles may be derived from the liver, the spleen and the pancreas or the gastric mucosa, the extract from which is used for proteolysis. In a sufficiently concentrated form they may be efficacious even in those cases which have proved resistant to the best treatment adopted so far.

The beneficial clinical results especially the recession of the enlarged liver, obtained with choline chloride in the early stages of the disease indicates that the initial stage of the disease is one due to a fatty liver which is amenable to choline treatment. More than mere lipotropic or protein factors, however, appear to be necessary for therapeutic treatment in the later stages.

In view of all the above findings further experimental work should be directed towards the study of the active principles involved in the therapeutic action of the glandular hydrolysate, their preparation, concentration and identification. The clinical work should be concerned with the therapeutic action of such preparations at various stages of the disease, both when given alone and along with lipotropic factors and protein hydrolysates. We have already started some preliminary work on preparations from the gastric mucosa.

Our clinical studies with protein hydrolysate have been confined so far to oral administration of these hydrolysates. Parenteral administration of these deserves a fair trial, especially in the advanced stages of the disease where oral alimentation may be defective.

Biochemical studies on protein and fat metabolism should be conducted at various stages of the disease. Since beneficial clinical responses to choline and protein hydrolysates have been obtained in the early stages of the disease, a study of the plasma levels of phospholipid-choline, cholesterol, fatty acids, and amino acids particularly the sulphur-amino-acids, at various stages of the disease and after treatment, and also in normal children within the appropriate

age limits, would throw much light on their mode of action. These studies will help to formulate the exact biochemical defects pathognomonic of the disease and provide the necessary biochemical background for tracing the evolution of the disease.

The liver biopsies should be extended to the earlier stages of the disease.

Summary of work done.—The work done so far and the tentative conclusions drawn with regard to the specific aspects assigned for investigation under the enquiry may be briefly summarized as follows :—

(a) Choline chloride has been found to be effective in the early stages of the disease. Its therapeutic action is slower than that of a pepsin proteolysed extract of the liver and spleen supplemented with choline chloride. Further clinical work on the action of choline chloride is in progress.

(b) The thymol-turbidity test of liver function has been found to be of diagnostic and prognostic value in conjunction with clinical symptoms in this disease. The methylene blue test is of no value for clinical diagnosis and prognosis. Comparative studies of various liver-function tests—the thymol-turbidity, the cephalin-cholesterol, and bromsulphalein-excretion tests—are being conducted.

(c) Liver biopsies have been performed in two cases in the advanced stage of the disease. The biopsy specimen revealed on histological examination diffuse necrosis and fatty degeneration. No pathogenic organisms were detected in these specimens. Further experimental data is necessary for drawing any conclusions on this aspect.

9. Dental research unit under Dr. C. D. Marshall Day at the deMontmorency College of Dentistry, Lahore.

The work of the Dental Research Unit, I.R.F.A. at the deMontmorency College of Dentistry has been continued under the direction of Dr. C. D. Marshall Day.

The scheduled field research of the unit was seriously interfered with on account of the communal disturbances in the Punjab, but other investigations were proceeded with so far as circumstances permitted. Some of the work undertaken has been completed but of some problems yet remain to be finished. It is hoped that these will eventually be brought to a state of completion.

In view of the fact that Dr. Marshall Day proceeded on leave pending retirement it was found necessary to close down the unit on 31st August, 1947.

During the limited period between 1st April and 31st August it was found possible to finalize certain investigations begun last year.

Other investigations along similar lines which are being carried out by Mr. Baij Nath Mehra, M.D.S., Mr. Ijaz-ul-Haq, M.D.S., and Miss Vimla Sud, B.D.S., are in various stages of completion and should be completed as soon as the present disturbances permit.

The efforts of the unit were mainly concentrated upon a radiological survey of periodontal disease in India. Following are the findings of the major portion of the scheduled survey so far completed :—

Following earlier reports on clinical studies and a recent report on the incidence of hypertrophic gingivitis, the present investigation outlines the results of a radiological survey of periodontal disease in Northern India. Emphasis is placed upon the importance of this disease in internal medicine and as a public health problem.

X-ray examination of a total of 568 subjects from 9 to 60 years of age established the fact that in the Indian subject periodontal disease, as manifested by actual bone resorption, has its incipiency in childhood and that after the age of 16 years bone resorption in varying degree is almost universal.

The average degree of bone resorption was found to increase with age, though the relationship was by no means constant for individuals. In all groups from 23 years and upwards subjects were found with alveolar bone resorption extending as far as the root apex in one or several areas. As much as half this depth of destruction was observed in a child of 13 years.

The bicuspid region of the alveolus was found to be most resistant, and the molar region, closely followed by the lower incisor area, the most susceptible to the process of resorption.

The horizontal or marginal type of bone resorption was observed in 66 per cent and the vertical of diffuse type in 34 per cent of the subjects affected. The absence or imperfect structure of the alveolar bone cortex and lack of uniformity in bone density and texture in a high percentage of Indian subjects would seem to point to a condition of physiological bone weakness and consequent susceptibility to disease.

Marginal periodontitis, periodontosis and the syndrome of periodontosis and marginal periodontitis were found to occur in the ratio of 65·13, 17·57 and 17·30 per cent, respectively. A study was also made of the incidence of the slow, rapid and arrested types of bone resorption.

A comparison of sex incidence suggests a lower incidence and average degree of alveolar bone resorption and a relatively higher incidence of the vertical type of resorption in the female subjects.

The extremely high incidence of periodontal disease of all types, its frequent occurrence at a very early age, the high percentage of subjects with periodontosis or the syndrome, the absence or imperfect calcification of the cortical layer, the frequent lack of uniformity in density of the alveolar bone and the comparatively advanced degree of bone dissolution so frequently encountered in Indian subjects even in the younger age groups would seem to suggest a systemic background for periodontal disease in India.

Recent scientific investigation has emphasized the marked effect on the periodontal tissues of deficiencies of minerals such as calcium and magnesium, of the vitamins A, B and C of endocrine dysfunction and other metabolic disorders. In view of the known tendency towards nutritional deficiencies and the high incidence of metabolic disturbances in this country, future research in relation to the ætiology of this most common of all diseases might well be conducted along these lines.

10. Enquiry on the relationship between the measurements of vertebræ and body-size of Indians under Dr. R. G. Ginde at the Seth G. S. Medical College, Bombay.

The subject of spinal tumours is extremely interesting to the patient and medical men alike, as the results of surgery in the vast majority of cases are so

gratifying that a person condemned to lead a life worse than a vegetative one is helped back to lead a healthy normal life with pursuits of happiness. Such an end-result would only be possible when a space occupying lesion in the spinal canal happens to be, as often it is, of a benign nature, and when these cases are detected early and are removed surgically. The first important step towards this end is, therefore, early diagnosis. As an aid to this, a number of investigations, from simple to more complicated have to be carried out. Measurements of interpediculate distances of the vertebræ have been found to be very helpful and at the same time a very simple method of investigation in the early diagnosis of such a case. There are two varieties of interpediculate distances horizontal and vertical, the former is more often useful, as is also found in our very limited experience. In America C.A. Elsberg and J. D. Camp have studied these in greater detail and the former with Dyke has published a chart of the average and maximum measurements of the horizontal interpediculate distances in mm. in normal American subjects above the age of 12. This chart is taken as the normal to compare with the corresponding figures in a spinal-tumour suspect, to find if there is any localized increase of the H.I.D. especially 4 mm. or more above the maximum normal.

With the gradual increase of interest in this subject it was found that the American figures were bigger than the figures obtained in some of our proved spinal-tumour cases. Therefore, with a view to find out normal figures among Indians, Hospital and other *x*-rays of the normal and diseased spines of all adults were measured in mm. similarly to get an average from a larger number, and to see if a marked difference exists, the same measurements were taken from the spines, seen in *x*-rays taken for suspected lesions of the urinary tract and abdomen. As the average measured in some regions, showed a discrepancy of over 4 mm. a size quite sufficient to produce enough pressure symptoms on spinal elements it was decided to study this problem more scientifically and a grant was asked for, from the Indian Research Fund Association.

Material and method for study.

Mostly the students of Seth G. S. Medical College were selected. Some nurses, doctors, and patients were taken to complete the number 150. Out of 150, 100 are males and 50 are females. Their ages are between 16 and 47 with average of 22·18, 90% being between the ages of 18 and 26.

The following measurements were taken: (1) Total height, (2) tragus height, (3) cranial height, (4) sitting height and (5) vertebral height.

X-rays of the same subjects were taken using the same technique namely—the *x*-ray plate was kept 36 inches away from the cathode using Potter Buckey diaphragm. For cervical spine, 15 degrees tilt towards the head of the person was given and the head was kept extended. Dorsal and lumbar spines were taken with the standard technique. The horizontal interpediculate distances were measured in mm. with a vernier caliper.

The average, maximum and minimum figures were worked out with other specification as shown in the charts. Cadavers were *x*-rayed, macerated and actual measurements were taken from dried bones. Care was taken to see that each cadaver was macerated in a separate chamber so that the vertebræ did not get mixed up. By courtesy of the department of anatomy, Seth G. S. Medical College, measurements were taken from the vertebræ obtained from 8 such cadavers and further 4 additional cadavers, which were first *x*-rayed and then macerated, each in a separate chamber. This latter procedure was

done to find out the degree of magnification of the actual measurements that occurs in the x-ray measurements. No definite conclusions can be drawn from such a small series of figures, however, the results are suggestive, requiring further work to conform or draw definite conclusions from them.

Though the number of persons is 150, the total number of vertebral measurements is not 150 throughout the series, because some of the upper cervical vertebræ could not be measured owing to overlap of the mandible and skull shadows; or the margins being not very clear. Similarly, in the lower dorsal region a few missing numbers are due to 11 thoracic vertebræ and partly due to not being able to measure a few x-rays accurately. The last lumbar figures are less, because of sacralization of the 5th lumber vertebræ.

The above work was undertaken with a view to find out:—

(1) A standard of H.I.D. of the vertebræ amongst Indian subjects for comparison with the measurements in spinal tumour suspects, as a diagnostic aid.

(2) To find out the degree of magnification, if any, that occurs in x-rays from the actual bony measurements.

(3) To find out the relation between the H.I.D. and the length of the vertebral column and the approximate height of the individual. This latter requires further study before definite conclusion could be drawn.

11. Enquiry into the causes of blindness under Major E. J. Somerset at the Medical College, Calcutta.

The work commenced on 15th April, 1946 and an interim report of the first one thousand cases was published in the *Report of the Scientific Advisory Board* for the year 1946, page 80. The extremely unsettled conditions in Calcutta from August 1946 to August 1947, caused a great drop in the out-patient attendance at the Eye Infirmary. In spite of these great difficulties, we have now collected 3,000 cases of uniocular and binocular blindness and recorded their details in the same manner as the first 1,000 cases. We are again indebted to Professor Chandra Sekhar for transferring our results to the special cards and analysing them with the Powers-Somas (Sorting) Accounting Machine at the All-India Institute of Hygiene & Public Health, Calcutta.

Tables I, II, III and IV show some of the ways in which the first 2,000 cases have been analysed statistically. The enquiry will finish in April 1948, when a much more detailed and elaborate analysis will be given, and the main groups will be broken up into sub-groups showing details of specific causes.

TABLE I.

Age:— / Sex.	0–1	1–4	5–15	16–29	30–39	40–49	50–59	60– over	Total
Male ..	18	34	130	225	198	205	308	317	1,435
Female ..	8	16	52	52	40	79	144	174	565
Total ..	26	50	82	277	238	284	452	491	2,000
Percentage	1·30	2·50	9·10	13·86	11·91	14·16	22·61	24·56	

TABLE II.

Which eye affected.

Right eye.	Left eye.	Both eyes.	Total.
687	619	694	2,000

TABLE III.

Cause of blindness	Right eye.		Left eye.		Both eyes together.		Both eyes—same case.	
	No.	% of total.	No.	% of total.	No.	% of total.	No.	% of total.
Congenital and undetermined	365	53·13	317	51·21	682	52·22	391	60·81
Exogenous infection	156	22·71	137	22·13	293	22·43	96	14·93
Endogenous infection	93	13·54	83	13·41	176	13·48	83	12·91
Traumatic and chemico-toxic	64	9·32	68	10·99	132	10·11	7	1·09
Systemic diseases	9	1·31	14	2·26	23	1·76	65	10·11
Not classified	..	..	..	..	..	..	1	0·16
Total	687	..	619	..	1,306	..	643	..

TABLE IV.

BOTH EYES.

L. Eye R. Eye	Congenital and undetermined	Exogenous infection	Endogenous infection	Traumatic and chemico-toxic	Systemic diseases	Not classified	Total
Congenital and undetermined	391	15	3	3	..	..	412
Exogenous infection	7	96	7	..	..	..	110
Endogenous infection	2	7	83	..	..	..	92
Traumatic and chemico-toxic	5	1	..	7	..	..	13
Systemic diseases	..	..	..	..	66	..	66
Not classified	..	..	..	..	..	1	1
Total	405	119	93	10	66	1	694

12. Enquiry on typhus under Dr. D. W. Soman at the Haffkine Institute, Bombay.

I. Work on Typhus in Bombay.

The investigation undertaken was mainly concerned with the serological diagnosis of typhus fever by Weil-Felix reaction and isolation of rickettsial strains whenever possible. The non-specificity of the Weil-Felix reaction presented difficulties in the correct interpretation of the ætiological diagnosis of this disease, the reaction being of very limited value in distinguishing with certainty between louse, flea and tick typhus. Of recent years, additional methods of approach to this problem has been afforded by the preparation of specific rickettsial antigens from infected mouse-lungs or chick-embryo yolk sac. These specific rickettsial antigens were available in limited supplies through the courtesy of American and Canadian workers and it was possible to use them in the present study.

During the period under review, serological reactions such as Weil-Felix, rickettsial-agglutination and complement-fixation tests were carried out on human sera. Isolation of strains from cases, rats and mites were attempted. Besides, complement-fixation test was carried out on rat sera to assess the incidence of rickettsial infection in them.

(a) *Serological diagnosis.*—Sera from 1,182 human beings were examined by Weil-Felix reaction and 85 of them were found positive for *B. proteus* OX19 antigen in a titre of 1 in 250 or over. None of these samples gave positive results with *B. proteus* X2 antigen. OX19 titre in different cases varied from 1 in 250 to 1 in 3,200 and no correlation was observed between the magnitude of the titre, the day of disease, or the severity of infection. Out of 55 cases, clinical history was available in 22 for study. On the whole, the course of typhus infection (Murine type) was mild with rash and delirium only in 20% of cases. Such cases were clinically indistinguishable from mild enteric infections except by laboratory tests.

(b) *Isolation of strains.*—Eight attempts were made by inoculating male guinea-pigs intraperitoneally with patient's blood with success in one. This guinea-pig showed febrile reaction with no orchitis and scrotal swellings; later its blood serum fixed murine rickettsial antigen. The difficulty of isolation of Murine strains from patients has been recognized by different workers and is attributed chiefly to the presence of strains of low virulence. As previously reported 27 attempts were made to inoculate guinea-pigs and only in four cases *R. mooseri* could be demonstrated.

(c) *A preliminary study of complement-fixation and rickettsial-agglutination tests in typhus.*—Complement-fixation tests were carried out using Bengston's technique, on 90 W. R. sera and all of them were found to be negative, in a dilution of 1 in 5, which was taken as the minimum diagnostic titre. Eighty-eight samples derived from 58 cases, suspected of typhus fever, were tested by this method and 35 cases were diagnosed as murine or flea-borne typhus, 3 as epidemic or louse-borne typhus and 3 cases provisionally as tick typhus. Seventeen cases which showed OX19 titres of 1 in 100 and 1 in 200 were found to be negative. Sera from two epidemic cases were received from Gharwal District in which louse-borne typhus has been reported. In two cases the sera fixed the Rocky Mountain Spotted Fever antigen in a titre of 1 in 5 and 1 in 10. As pointed out before Weil-Felix reaction is valueless in distinguishing one type from another.

The rickettsial-agglutination reaction was also carried out by using murine and epidemic rickettsial suspensions taking 1 in 100 as the diagnostic titre. In the majority of sera tested, cross-fixation usually occurred but the titres against the homologous strain were always higher. In all 79 samples were tested derived from 53 cases. On the whole, the results of complement-fixation and rickettsial-agglutination tests, ran parallel because majority of serum samples tested were collected after the 10th day of disease.

The Table is representative of the general results:—

TABLE.

No.	W.F.		Rickettsial-agglutination		Complement-fixation			Nature of typhus infection
	OX19	OX2	M	E	M	E	R.M.S.	
1	400	..	100	100	10	..	..	Flea-borne or murine.
	800	..	400	400	40	..	..	
2	250	..	50	800	5	40	..	Louse-borne or epidemic.
3	250	..	200	400	20	40	..	—do.—
4	400	..	..	..	..	..	10	Tick-borne
5	100	..	..	..	..	..	5	—do.—

II. WORK ON SCRUB TYPHUS (MITE TYPHUS).

(a) As previously reported, the presence of scrub typhus in Bombay City was definitely established by isolation of *R. orientalis* from human cases in experimental mice. A new field for investigation was soon open to establish a causal arthroped vector and the animal reservoir of infection. Sera from 1,182 human cases were tested for Weil-Felix reaction using *proteus* XK antigen and 13 of these were found to be positive, these were derived from 5 cases. These occurred in the months of January, February, August and September. Unlike the other varieties of typhus the Weil-Felix reaction using XK antigen was quite specific in the diagnosis of this infection. Rash was present only in 2 cases and the pathognomic eschar was absent in all.

(b) *Isolation of human strains.*—Rickettsial isolation was attempted in all 5 cases and only in 2 cases it was successful. In both the cases mice passages had to be repeated 3 to 5 times to establish the strain and to demonstrate *R. orientalis* in peritoneal exudate. Some of the local strains isolated last year were studied by cross immunization experiments in rabbits using intra-occular mode of infection. Sera of these rabbits were further tested by Weil-Felix reaction with negative results.

(c) *Isolation of mite strains.*—Six attempts were made to inoculate mice with the suspension of crushed larval mites numbering 50 to 100 collected from Bombay rats. Mite collection was carried on at random during the months of February, August and September. All the attempts proved negative; no mite-strain

could be isolated. The arthropod vector and the rodent reservoirs remain yet to be established to complete the epidemiological studies of scrub-typhus infection in Bombay. The work of systematic collection of mites from rats and their identification and isolation of mite strain forms the subject for intensive study next year.

III. Complement-fixation Test on Sera from Bombay Rats and Isolation of Rickettsial Strains.

(a) Three species of Bombay rats were taken for study, *Rattus rattus*, *Rattus norwegicus* and *Gunomys*. With a view to determine the incidence of rickettsial infection, complement-fixation tests using murine antigen were carried out on sera of 384 rats, collected during the months of January to August of this year. Ninety-nine rat sera (25·8 per cent) were found to be positive in a titre of 1 in 5 or over. Among the three species *Rattus norwegicus* showed the highest incidence of rickettsial infection. In the epidemiological study, the serology of rodents can only be investigated by the complement-fixation and rickettsial-agglutination tests, as Weil-Felix results have been found to be unreliable.

(b) *Isolation of rickettsial strains.*—Male guinea-pigs were inoculated with pooled brain suspensions of 3 to 4 rats of the same species for isolation of strains. Seven attempts were made and one rat-strain was isolated. Two animals did not react, four showed only febrile reaction and one showed pyrexia with Neill-mooser reaction. *R. mooseri* was demonstrated in the tunica exudate. It was interesting to note that sera from rats whose pooled brains when inoculated into a guinea-pig showed Neill-mooser reaction, fixed the murine a antigen to a titre of 1 in 20.

13. Enquiry on typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta.

This enquiry is a continuation of the Pilot Enquiry on Typhus which was begun in October 1946 with a grant kindly placed at the disposal of the Department of Microbiology by the Director of the All-India Institute of Hygiene & Public Health, Calcutta.

The lines of work undertaken were :—

(1) Serological investigation of fever cases in the Barrackpore and Ichapore areas.

(2) Isolation, maintenance and cultivation of rickettsia.

(3) Study of rodents and their ectoparasites.

(4) Development of a suitable technique for breeding mites.

(5) Animal experimentation for determining the modes of transmission.

(1) *Serological investigation of fever cases.*—In addition to the serological examination of specimens whenever feasible the blood clot was also cultured to determine the presence of pathogens. Of 43 samples cultured, *B. typhosus* was isolated from 5 ; *Paratyphosus* A from 1 and *Staphylococcus aureus* from 1 ; 33 were sterile and 3 contaminated.

The total number of samples examined was 120. Of these there were :—

Typhus cases	..	22 (OX_{19}—4 ; OX_2—1 ; OXK—17)
Typhoid cases	..	17
Paratyphoid cases	..	2
Kala-azar cases	..	7
Staphylococcal septicæmia		1
		49

The clinical picture of typhus cases in this area among the indigenous population were the same as stated in the report of the Pilot Enquiry, that typical signs and symptoms of typhus, such as eschars, rash, early mental confusion and adenitis are seldom, if ever, seen.

The popular method for diagnosing typhus is by the Weil-Felix test, but it is felt that more reliable results would be obtained by complement-fixation or agglutination tests with local strain of rickettsia. Agglutination in low dilution was found with many of the sera from undiagnosed cases and in one instance a sample of blood which gave completely negative results on two occasions was found by mouse inoculation done with a portion of the 1st sample of blood to be a case of typhus XK. The mouse was very sick on the 11th day and was sacrificed. Post-mortem the typical pathological features of ascites, enlarged spleen and rickettsia in the endothelial cells were present. The inoculation of mice with a small quantity of blood washed out of the clot is being done in all suspected cases as a routine measure.

(2) *Isolation, maintenance and cultivation of rickettsia.*—Three strains of rickettsia from brains of rats and three from human cases have been isolated and are being maintained in white mice.

Bengal strains of typhus XK are very avirulent to guinea-pigs and give hardly any febrile reaction after the 3rd or 4th passage. It is more virulent to mice which succumb about the 12th day to an inoculation of washed blood cells of typhus patients or emulsions of spleen or brain from infected animals.

The rat strains were isolated by the inoculation of brain emulsions of wild rats which harboured *T. deliensis*. Of 21 rats brains inoculated three infections resulted. It is unfortunate that larvæ or nymphs from these animals were not crushed and inoculated into other mice at the same time, as the inoculation of 8 batches of nymphs from other rats did not produce a single infection.

The cultivation of rickettsia in the yolk sacs of developing chick-embryos is being done, but up to date no definite results have been obtained. One drawback is the inability to obtain a sufficient number of fertile eggs at the present time.

(3) *Study of rodents and their octoparasites.*—Rodents were collected mainly from two areas in Barrackpore and Ichapore from where typhus cases were known to have occurred during the past two years. Up to date a total of 508 rodents have been captured and examined. Of these there were :—

R. rattus	..	414
G. varius	..	55
N. bandicoota	..	5
Shrews	..	32
Squirrels	..	2
		508

Thrombiculid mites were present in 173 of them in lesser or greater numbers. During April and May the number of infested animals was small compared with later months, when both the number of infested animals and mites were much greater, as will be seen from the figures below :—

Month.	Number of rodents.	Number infested.
April ..	8	1
May ..	163	24
June ..	72	25
July ..	41	11
August ..	86	60
September ..	94	37
October (up to 20th)	44	15

One or more mites were mounted from each infested animal and of 288 slides examined. *T. deliensis* was present either as a single or mixed infection in 136 of 173 infested animals.

Shrews (*Crocidura coerulea*) appear to be a favourite host of *T. deliensis*. Of 32 examined 24 were found harbouring *T. deliensis*. A site of election for *T. deliensis* is the outer sides of the fore and hind legs of these rodents as well as the bases of their tails—fairly large numbers being hidden among the hairs on these parts. Infestations in the ears are more often with *S. bengalensis* with a few *T. deliensis* on occasions.

G. varius does not seem to attract *T. deliensis*. Of 55 of this species examined 8 showed infestations of *S. bengalensis* and only one had a mixed infestation of *T. deliensis* and *S. bengalensis*.

Young rats were seldom found infested ; this is suggestive that mites do not breed in nests of rats and that these ectoparasites get attached when they forage for themselves for food or water.

Both squirrels examined were found free of octoparasites. The species of *Thrombiculids* collected were : (1) *T. deliensis*, (2) *T. muris*, (3) *T. munda*, (4) *T. schuffneri*, and (5) *S. bengalensis*.

In addition to the above species two of which *T. munda* and *T. schuffneri* were first reported from Malaya—two other species have been found which apparently have not been described before. These will be reported on later.

(4) *Development of technique for breeding mites.*—To obtain engorged larvæ for breeding, rats found to be harbouring appreciable numbers of mites are placed in small square wire mesh cages which are stood over a dish containing water ; when the larvæ become engorged they drop off the rat and are found floating on the water from where they are collected.

Day-to-day collection of larvæ from the rats showed that *T. deliensis* may remain attached for 6 days ; *T. muris* for about 24 days and one of the new species also a small mite for about 14 days.

Engorged larvæ are placed in specimen tubes containing moist sand and in 24 to 48 hours they are seen to get quiescent on the sand or against the glass ; in that condition they remain for 7 to 10 days before emergence of the nymphs.

Larval mites that are not engorged, such as those collected from the oars of shrews (which cannot be kept in captivity) also attain the nymphal stage if kept in the manner described above, but the nymphs are much smaller and begin emerging in about five days.

As will be seen the nymphal stage is very easily secured, but a technique for rearing adults from nymphs has still to be reported. The Japanese workers who claim to have reared a few adults from the larval stage state that nymphs and adults feed on potato, melon, roots of grasses and a species of daisy.

The duration of the nymphal stage also is unknown, as well as the number of nymphal instars before development into an adults.

Nymphs of *Thrombiculids* are armed with claws on their legs and pedipalps and have well-chitinized cholicerœ. With such an armature it may be expected that they may feed on solids or liquids obtained by piercing stems of grasses or roots or fruits. All attempts, however, to rear nymphs on potato or melon, or grasses of various kinds including sugar-cane, proved a failure.

In case nymphs might feed on other larvæ found in soil, attempts were made to rear them in soil containing sandfly larvæ, or worms or grubs, with no success.

In a tube containing nymphs it was observed that they were attracted by some moist silt which was present with the sand; more liquid river silt was added and it was found that they were able to get some nourishment from the silt. A few were alive after 20 days but the majority died in about 10 to 14 days.

Attempts to enrich the bacterial flora of the silt by the addition of organic matter such as dried blood, crushed liver and dead insects was of no use—in fact the nymphs died off very rapidly in a day or two at most.

At present attempts are being made to feed the nymphs on silt enriched with straw, cabbage or grass infusions, containing large numbers of bacteria as well as various *ciliates* and *verticella*.

The result of these experiments will be reported later.

Miscellaneous.—Two attempts to get unengorged larvæ collected from ears and legs of shrews to reattach to white mice and rats were not successful.

To complete the programme of work the following have yet to be done:

(1) A suitable technique for breeding mites.

(2) Transmission of infection by mites from infected to uninfected rodents.

(3) Cultivation of rickettsial strains in the yolk sacs of developing chick embryos.

14. Enquiry on the effect of vitamins C and B in diabetes under Dr. S. Banerjee at the School of Tropical Medicine, Calcutta.

Nicotinic Acid in Diabetes.

Contradictory claims have been made by several workers regarding the usefulness of nicotinic acid in the treatment of diabetes. The urinary excretion of nicotinic acid and the effect of intravenous injection of nicotinic-acid

amide on the blood-sugar and blood-acetone bodies of diabetic patients and normal subjects were, therefore, studied. The effect of intravenous injection of nicotinic acid on glucose tolerences test of normal rabbits, on the blood-sugar of normal and diabetic rabbits and on the urinary excretion of sugar by diabetic rabbits were also studied.

Estimation of nicotinic acid in the urine of diabetic patients.—In the chemical estimation of nicotinic acid in the urine by Konig's reaction, the urine is digested in a water-bath either with acid or with alkali in order to convert derivatives of nicotinic acid excreted in the urine into nicotinic acid. The same procedure when applied to the urine of diabetic patients gave a very highly coloured extract due to the sugar present in the urine. The interfering colour could not be removed by using any of the methods so far suggested by various workers. This problem was solved by treating the urine before digestion with potassium permanganate. The method of estimation of nicotinic acid developed in this laboratory is as follows.: To 25 c.c. of urine in a beaker is added 8 c.c. of conc. hydrochloric acid. The beaker is heated in a water-bath and is treated with 10% $KMnO_4$ until all the sugar is oxidized (1 g. sugar requires 16 c.c. of 10% pot. permanganate). The manganese present in the solution is removed as phosphate by treatment with disodium hydrogen phosphate and caustic soda. The solution is then digested in a water-bath for 45 minutes with 40% caustic soda so that the strength of the alkali is 4 per cent. The interfering colour is removed by making the solution acidic with concentrated HCl and treating it with $KMnO_4$ until the solution becomes almost colourless. The solution is adjusted to pH 7 and phosphate buffer is added (6 c.c. buffer per 10 c.c. of the solution). An aliquot of the solution is then treated with 1 c.c. of 4% alcoholic aniline and 6 c.c. of freshly prepared cyanogen bromide solution and compared in a colorimeter with a solution of standard nicotinic acid similarly treated. Blank correction is not necessary in all cases. Nicotinic acid added in the urine can be quantitatively recovered by this method.

Twenty-four hours' urinary excretion of nicotinic acid was estimated by the above method in seven diabetic patients which was found to vary between 2·62 mg. and 5·2 mg. These figures fall within normal limits as shown by Ghosh (*Ann. Biochem. Exper. Med.*, **1**, p. 235, 1941). Diabetic patients, therefore, do not seem to suffer from nicotinic-acid deficiency.

Effect of injection of nicotinic-acid amide on the blood-sugar of diabetic patients and normal subjects.—Five hundred mg. of nicotinic acid amide in a 10% solution were injected intravenously and samples of blood were taken both before and at intervals of half hour up to 2 hours after the injection. Blood-sugar was determined in the different samples of blood and the results are given in Table I:—

TABLE I.

Subject.	No. of cases.	Fasting blood-sugar (average) mg. %	Blood-sugar after injection of nicotinic-acid amide.			
			½ hour.	1 hour.	1½ hours.	2 hours.
Diabetic ..	16	273±24	272±24	266±23	269±25	274±26
Normal ..	5	98	101	106	104	103

Nicotinic-acid amide does not seem to have any effect on the blood-sugar of normal and diabetic subjects. The claims of Neuwahl (*Lancet*, **2**, p. 349, 1943) that nicotinic acid lowers the blood-sugar to produce hypoglycemic convulsions could not be confirmed.

Effect of nicotinic acid on the blood-acetone bodies of normal and diabetic subjects.—Five hundred mg. of nicotinic-acid amide were injected intravenously and samples of blood were taken both before and at half-hourly intervals up to two hours after the injection of nicotinic-acid amide. Blood-acetone bodies were determined and the results are shown in Table II :—

TABLE II.

Subject.	No. of cases.	Fasting blood-acetone bodies (average) mg. %	Blood-acetone bodies after injection of nicotinic-acid amide.			
			½ hour.	1 hour.	1½ hours.	2 hours.
Diabetic ..	10	6·01	8·05	10·65	8·50	8·57
Normal ..	3	0·91	0·93	0·95	1·13	1·41

Nicotinic-acid amide does not seem to have any effect on the total acetone bodies level of blood of both normal and diabetic subjects.

Effect of nicotinic acid on the blood-sugar level of normal and diabetic rabbits.—Three rabbits were made diabetic by intravenous injections of alloxan which specifically destroys the beta-cells of the islets of langerhans of the pancreas. After an overnight fast three normal rabbits and the three diabetic rabbits were intravenously injected with 50 mg. of nicotinic acid in a 2 per cent solution and samples of blood were taken before and at definite intervals up to 225 minutes after the intravenous injection of nicotinic acid. Blood-sugar values are given in Table III :—

TABLE III.

Rabbits.	No. used.	Fasting blood-sugar (average) mg. %	Blood-sugar after injection of nicotinic-acid amide.			
			45 mts.	105 mts.	165 mts.	225 mts.
Normal ..	6	110	194	144	118	113
Diabetic ..	3	386	463	528	506	..

Nicotinic acid seems to increase the blood-sugar of diabetic rabbits and it has very little effect on the blood-sugar of normal rabbits.

Effect of nicotinic acid on glucose tolerance test of normal rabbits.—Ten healthy male rabbits were fasted for 24 hours. After the fasting blood samples were taken all the rabbits received an intravenous injection of a 50 per cent solution of glucose (1 g. per kilo). Five of the rabbits received in addition 50 g. of nicotinic acid which was injected intravenously just after the injection of

glucose. Samples of blood were taken at definite intervals up to 225 minutes after the injection of glucose. Results are shown in Table IV:—

TABLE IV.

Rabbits.	No. used.	Fasting blood-sugar (average) mg.%	After injection of glucose or glucose and nicotinic acid.			
			45 mts.	105 mts.	165 mts.	225 mts.
Injected with glucose only ..	5	124	269	166	112	110
Injected with glucose and nicotinic acid..	5	115	255	154	111	110

Injection of nicotinic acid did not alter the glucose tolerance curves of rabbits.

Effect of nicotinic acid on the urinary excretion of sugar by diabetic rabbits.— Effect of intramuscular injections of 50 mg. nicotinic acid on the urinary excretion of sugar by rabbits was also studied. Results are shown in Table V:—

TABLE V.

Number of rabbits.	24-hours urinary excretion of sugar.	
	Before nicotinic acid.	After nicotinic acid.
3	4·7 g.	4·4 g.

Injections of nicotinic acid did not appreciably alter the urinary excretion of sugar in diabetic rabbits.

From the above studies it may be concluded that nicotinic acid has no place in the treatment of diabetes.

15. Survey of hookworm diseases in Jharia Coalfields Settlement Area under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

The work has been started from 1st September, 1947 in a central laboratory located in the office of the Jharia Mines Board of Health at Dhanbad and it is expected that the work will be finished by March 1948 when a report will be published.

16. Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

The works carried out in the present year were mainly concerned with assessing the value of synthetic folic acid (Lederle) in the different types of an anæmia; the hæmopoietic activity of the drug in other disorders of blood was

also investigated in a few cases. The effect of folic acid on 'sprue syndrome was also studied in a few cases.

Folic acid was always given orally in doses varying from 10 mg. to 30 mg. a day and unless otherwise mentioned it was always given for 30 days.

A. Action of Folic Acid on Anæmias.

During this period 62 cases of anæmia were treated with folic acid of which 30 were cases of macrocytic anæmia, 2 were cases of microcytic hypochromic anæmia and 30 were cases of anæmia in pregnancy.

(i) *Macrocytic anæmias.*—On the basis of the hæmoglobin concentration in the red cells and bone-marrow findings the macrocytic anæmias were further subdivided into the following groups :—

(a) Macrocytic anæmia with megaloblastic reaction of the marrow.

(b) Macrocytic anæmia showing generally at least a few megaloblasts in the bone-marrow but having a low M.C.H.C. (dimorphic anæmia).

(c) Macrocytic anæmia with hormoblastic reaction of the marrow.

(d) Macrocytic anæmia with hypoplastic bone-marrow.

(a) *Macrocytic anæmia with cellular megaloblastic marrow.*—Fourteen cases. Blood picture reached almost the normal level in 11 cases, showed moderate improvement in 2 cases and only slight improvement in 1 case.

With 30 *mg. dose*—10 *cases.*—Eight cases showed optimal improvement, 1 showed moderate improvement and 1 showed only slight improvement. One of the cases showing good improvement with 30 mg. did show only slight improvement in the initial stages with 20 mg. but the improvement rate was speeded up when the dose was increased to 30 mg. The case showing only slight improvement did not show any further improvement later either with liver or iron. In the 8 cases showing good improvement, the improvement in the blood picture was preceeded by moderate to good reticulocyte response in 3 (including the case receiving 20 mg. in the initial stage), tolerable response in 2 and only slight response in 2 while slight reticulocyte response was noted in the case showing moderate improvement, moderate reticulocyte response was seen in the cases showing only slight final improvement.

With 20 *mg. dose*—2 *cases.*—One case showed good improvement and another moderate improvement with 20 mg. a day for 30 days. Improvement was preceded in both the cases by moderate reticulocyte response. The case showing moderate improvement showed further improvement later on, with marmite. As already mentioned one case showed only slight improvement preceeded by moderate reticulocyte response, with 20 mg. dose but showed good improvement when the dose was raised to 30 mg.

With 10 *mg. dose*—2 *cases.*—Both the cases receiving 10 mg. of folic acid, showed good improvement and the improvement was preceeded by moderate reticulocyte response in one while no reticulocyte response was seen in the other.

Of the 13 cases showing improvement 7 presented themselves for a second examination within about a month after discharge from the hospital and each of these showed some improvement in the blood counts.

The optimal response was obtained in 2 cases with 10 mg. and in other cases with bigger dosage, in one case with 20 mg. produced only slight

response but increase of the dose to 30 mg. produced the optimal response. It will be seen that the initial reticulocyte response did not give any correct indication of the final improvement shown by the different cases. But the reticulocyte response was generally of a higher order than what is usually seen after liver therapy. These results are in conformity with our preliminary report published in the *I.M.G.* (October 1946).

(b) *Macrocytic anæmia with low M.C.H.C. but showing at least a few megaloblasts* in the bone-marrow (the cytoplasm of late megaloblasts was found to be deeply basophilic in a few cases) (? dimorphic anæmia).

There were 10 cases in this series. Two of these cases had an attack of malarial fever, one with plasmodium vivax after 25 days' treatment with folic acid and the other with *Plasmodium falciparum* after 15 days' treatment with folic acid. The first patient with benign tertian malaria showed considerable improvement of blood picture, with moderate degree of reticulocyte response, before the development of malaria. After treatment of malaria with paludrine slight lowering of blood picture was noted which again improved with a 2nd course of folic acid. In the 2nd case with a huge enlargement of spleen and with malignant tertian malaria, the blood picture showed only slight improvement with folic acid, but treatment of malaria with paludrine not only cured malaria but helped to improve the blood picture as well; further treatment with tonic mixture, containing a good amount of ferrous sulphate brought the blood picture almost to the normal level. In the remaining 8 cases the response was as follows :—

With 30 *mg. dose—5 cases.*—The response was good in three and moderate in two cases. The improvement was preceded by moderate to good reticulocyte response in all the cases showing good improvement and also in one of the cases showing moderate improvement. While slight reticulocyte response was seen in the other case showing moderate improvement. In one case showing good improvement the dose was reduced to 10 mg. after 15 days, without in any way affecting the improvement rate. One of the cases showing moderate improvement showed further improvment with liver.

With 20 *mg. dose—2 cases.*—The response was moderate in one without any reticulocyte response and tolerable in the other with slight reticulocyte response. The case showing moderate improvement did not show further improvement with liver given after folic acid but the other case showed considerable improvement with iron given after folic acid.

With 10 *mg. dose.*—The only case with 10 mg. showed good response with moderate reticulocyte response. The patient showed further improvement with iron given after folic acid.

The bone-marrow was not examined in one case and this case showed good improvement with 30 mg. of folic acid. The bone-marrow in the other 7 cases was megaloblastic. In this group there was better correlation between reticulocyte response and final improvement.

(c) *Macrocytic anæmia with normoblastic marrow.*—In the single case studied in this group, the blood picture reached the normal level with 10 mg. a day without any reticulocyte crisis. The improvement was sustained.

(d) *Macrocytic anæmia with hypoplastic bone-marrow with paucity of cells of the red cell series, which were predominantly normoblastic in type.*—There were 5 cases in the group. Of these 2 with red cell count below one million per c.mm. and receiving 30 mg. a day died within 15 days of folic-acid treatment. One

was treated in the hospital for over 2½ months with folic acid 30 mg. a day, other haematinics and transfusions of blood without any improvement. The patient was discharged at his own request and died within a month after leaving the hospital. The remaining two cases improved considerably, one with 20 mg. dose and the other with 30 mg. dose, but neither of these patients showed any reticulocyte response. One of the cases showed slight fall of the blood picture after discontinuation of folic acid, but the blood picture improved again with liver extract and transfusion of blood. The other case is still under observation.

(ii) *Hypochromic anæmia.*—Folic acid was given in 30 mg. dose to 2 cases of hypochromic anæmia, one a case of normocytic hypochromic anæmia with cellular normoblastic marrow and showing 11,200 hookworm ova per c.c. of stool for 15 days and the other case of microcytic hypochromic anæmia without showing any hookworm ova in the stool for 10 days without any improvement in the blood picture in either. Both these cases responded promptly to iron therapy.

(iii) *Pregnancy anæmia.*—Thirty cases were put under trial with folic acid, of these, 3 cases died, 2 on the 2nd day of treatment and 1 on the 11th day of treatment with folic acid—this last case showed slight improvement of blood picture done on the 10th day. The remaining 27 cases are divided into 2 groups.

Group A : The cases where the treatment was given during pregnancy—14.

Group B : The cases where treatment was started just before confinement or within a few days after confinement—13.

In the 1st group, 1 left hospital before completion of treatment and in the 2nd group, 4 left hospital before completion of treatment.

Group A : with 30 *mg. dose*—2 *cases.*—Both the cases showed good improvement. In one case sternal puncture was not done and this patient showed moderate reticulocyte response. The other case with megaloblastic marrow showed good reticulocyte response.

With 20 *mg. dose*—6 *cases.*—Sternal puncture was done in 5 of the 6 cases and in all these the bone marrow showed megaloblasts. The blood picture showed considerable improvement in one (with megaloblastic marrow), moderate improvement in four (3 with megaloblastic marrow and one in whom the sternal puncture was not done), and no improvement in one (with megaloblastic marrow). The reticulocyte response was good in all the 4 cases showing moderate improvement and was only slight in the case showing good improvement and also in the case not showing any improvement at all. The patient who did not show any improvement with folic acid had not responded to proteolysed liver extract given before folic acid but showed improvement with liver extract given parenterally.

With 10 *mg. dose*—6 *cases.*—One of the cases with slightly megaloblastic marrow left hospital on the 12th day before a 2nd count was done. This case showed only slight reticulocyte response, but clinically the patient was feeling better.

Good improvement was seen in one case with megaloblastic marrow, which was preceded by moderate reticulocyte response. Two cases with megaloblastic marrow and one case in which sternal puncture was not done, showed moderate improvement with moderate reticulocyte response, in the 2 cases with megaloblastic marrow. These 2 cases with megaloblastic marrow showed further improvement when the dose of folic acid was raised to 30 mg.

One case did not respond at all with folic acid. This case later showed slight improvement with liver.

Group B.—Four cases left hospital before completion of treatment. One of these cases with megaloblastic marrow showed considerable improvement within 12 days, with 10 mg. of folic acid.

With 30 mg. dose—5 cases.—Four cases with megaloblastic marrow showed good improvement which was preceded by moderate to good reticulocyte response in 3 cases, and slight reticulocyte response in 1 case. In one of these the dose was reduced to 20 mg. after 10 days without affecting the improvement rate. The 5th case receiving 30 mg. dose had cellular normoblastic marrow and did not show any improvement up to the 15th day of folic-acid treatment; later this case improved with iron.

With 20 mg. dose—2 cases.—Both the cases had megaloblastic marrow. One case showed good improvement and the 2nd case showed moderate improvement with slight reticulocyte response in both.

With 10 mg. dose—1 case.—Sternal puncture was not done in this case. There was no improvement up to the 17th day and the reticulocyte response was slight. Later on, when the dose was increased to 30 mg., the patient showed slight improvement after 8 days when the patient left hospital.

Besides this case another patient was treated with 10 mg. a day. This patient showed good improvement but left hospital on the 12th day as mentioned before.

B. Leucopoietic Action of Folic Acid.

Leucopoietic response of folic acid was studied in 2 cases with extreme leucopenia due to granulocytopenia—one with enlarged spleen and moderate degree of anæmia and the other, a case of kala-azar. Folic acid did not produce any increase in the leucocyte count in either.

Definite leucopoietic response was however noted in some of the anæmic cases. In the macrocytic anæmic cases, with improvement of anæmia, definite increase in the count was seen in most of the cases with low initial leucocyte count.

In the cases of anæmia in pregnancy the leucocyte response was as follows:—

In cases of group A, where folic acid was given during pregnancy, the leucocyte count was found to be increased in the majority of cases; it remained unaltered in some cases while in a few cases there was definite lowering of the leucocyte count.

In cases of group B, where folic acid was given within a day or two after confinement, the cases with high initial leucocyte count showed definite lowering and came down to the normal range while in the cases with initial leucocyte count with normal range, the count remained unaltered.

The variation in the leucocyte count was always due to variation in the neutrophils.

In two cases, mentioned under sprue syndrome, the relative and absolute eosinophil counts were very high, after the completion of folic acid therapy.

C. Effect of Folic Acid on Platelets.

In a case of leucocyte, purpura folic acid did not show any improvement in the platelet count or in the clinical condition.

D. Sprue Syndrome.

Of the cases only two had slight anæmia and five cases had moderate to severe degree of anæmia. The bone-marrow was megaloblastic in all the anæmic cases.

In the anæmic cases, blood picture showed good improvement in 3 cases with 30 mg. dose. One case did not show any improvement with 10 mg. but showed good improvement when the dose was increased to 30 mg. In the 5th case only slight improvement was noted with 20 mg. This case later on, showed some more improvement with liver extract.

Clinical improvement was good in all these cases, as also in the other 3 cases with slight anæmia.

After completion of folic acid therapy, in two cases, one with moderate anæmia and the other with slight anæmia, the relative and absolute eosinophil count was found very high. In the anæmic case, the high eosinophil count persisted for over 2 months after which the patient could not be followed.

E. Study of Plasma Protein in Nutritional Macrocytic Anæmia.

Total plasma protein and albumin globulin ratio was estimated by micro-kjeldahl method in 43 cases of nutritional macrocytic anæmia. In 31 cases, plasma protein was found to be low, (below 6·0 g. per cent) with albumin: globulin ratio normal in 16 cases and with the ratio altered in 15 cases. In the remaining 12 cases the total plasma protein was found to be above 6·0 g. per cent; but in 8 of these cases the ratio was found altered due mainly to the reduction of albumin fraction.

In 8 cases of nutritional macrocytic anæmia with hypoproteinæmia, the plasma protein was estimated periodically along with cytological examination of blood. With clinical and hæmatological improvement plasma-protein level gradually rose to normal level with normal albumin: globulin ratio in 5 cases. The total plasma protein rose to normal level but the ratio was still altered in 2 cases, while in 1 case the total plasma protein did not reach the normal level.

17. Enquiry on kala-azar under Dr. P. C. Sen Gupta at the School of Tropical Medicine, Calcutta.

The work of this enquiry was considerably interrupted by the disturbed conditions in Calcutta mainly and the resignation of the assistant research worker of the enquiry to some extent, during the period under review. The present incumbent, Dr. K. C. Basu Mallik, M.B. joined his duties in February, 1947.

I. Chemotherapeutic Investigations.

1. *Therapeutic trials.*—

(a) 4 : 4′-diamidine diphenoxy pentane (Pentamidine, M & B 800).

During 1946 a series of 27 cases of kala-azar was treated with this drug with fair immediate results. It was proposed to follow up these cases 6 months or more after the completion of their treatment in order to find out the permanent cure rate. But on account of the serious disturbances in the city, and

the consequent change of address due to migration of the population, it was possible to follow up very few cases and thus the trial of the drug in this series of cases was not complete.

It was decided to institute a fresh clinical trial in 1947 and so far 42 cases have been treated with this drug this year. The preparation used was the 10 per cent solution of pentamidine di-B-hydroxy-ethane sulphonate, and the individual doses were 3 mg. per kilo body-weight. The treatment consisted of two courses of 10 injections each of pentamidine separated by 8 to 10 days interval between the two courses of injections in most cases. The trial is being continued.

(b) An Indian preparation of sodium antimony gluconate containing 100 mg. of antimony per c.c. of the solution was tested in a series of 38 cases during the year 1946-47. The immediate results were fairly good. So far 4 cases have relapsed. The final follow up the cases is being instituted in order to find out the permanent cure rate.

(c) An oily suspension of sodium antimony gluconate was tried out in a few cases, but the injections proved to be more painful than those of the watery solutions of the drug.

2. *Animal experiments.*

Cumulative toxic action of heated solutions of stilbamidine.—These studies were completed. A summary of the results obtained is given below. A paper on this subject is under preparation.

Lungs.—Vascular congestion, hæmorrhages into the alveoli in some cases, collapse of the lungs and rupture of the alveolar spaces and the presence of blood clot inside the bronchioles in one case, these were the chief abnormalities noticed in the lungs.

Liver.—The changes noticed were: varying degrees of fatty and granular degeneration affecting mainly the peripheral region of the lobules to a greater extent than the central parts, scattered area of neorosis in some cases, irregular hæmorrhages in the substance of the liver in others and in a few cases collection of small mononuclear cells in the portal tracts.

Spleen.—No marked changes were seen except some degree of vascular congestion affecting mainly the splenic sinusoids and the spaces. Siderosis was a common feature.

Heart.—Heart showed degenerative changes, viz. loss of transverse striations and separation of the fibres and fragmentation. Interstitial hæmorrhages were noticed in some cases.

Kidneys.—Showed degenerative changes mainly affecting the tubules; tubular necrosis was seen in some cases, and in one specimen interstitial hæmorrhages were seen.

Suprarenal.—The medullary sinusoids were uniformly dilated with hæmorrhage in some cases. Degenerative changes and even necrosis was seen in the cortex in some cases.

Nervous system.—The tissues were stained for Nissl's granules and by the Weigart Pal method besides the routine iron hæmatoxylin eosin stain. No vascular changes were noticed in any specimen. Except in one rabbit that developed paraplegia, no signs of neuronic damage or degenerative changes in the nerve tracts were seen in any of the experimental rabbits. In this particular

rabbit that developed paraplegia, extensive neuronic degeneration affecting the anterior horn cells of the lumbar region of the spinal cord and the pons were seen. No degenerative changes were seen in the white matter of the spinal cord. No vascular changes or perivascular cuffing was noticed in this case. It is difficult to decide whether the changes were due to the drug or to polioencephalitis caused by a virus, as no inoculation experiment was carried out.

II. Biochemical Studies in Kala-azar.

The work on this subject will be recommended as soon as the biochemist (whose appointment was sanctioned during 1947-48) joins his duties.

III. The Complement-fixation Test for Kala-azar using Antigens prepared for certain Acid-fast Bacilli.

This test has been found to yield satisfactory results in the early diagnosis of kala-azar in India.

In order to determine the reactions obtained with sera from cases of kala-azar from Sudan a supply of 25 sera from proved cases of Sudan kala-azar was obtained from Dr. R. Kirk, of the Stack Memorial Medical Research Laboratories, Khartoum. In over 90 per cent of the sera a positive reaction was obtained, with the complement-fixation test. This result is not significantly different from what is obtained in Indian kala-azar, viz. 93 per cent positive reaction.

During the year, one case of eosinophilia out of 20 such cases gave a positive complement-fixation reaction. A doubtful reaction was more frequently obtained. It is a curious fact that 'false positive reactions' are obtained with the Wassermann test in a small proportion of cases of eosinophilia.

18. Helminthological enquiry under Dr. A. K. Mukerji at School of Tropical Medicine, Calcutta.

Continuous study of the problem which was begun last year was rendered impossible due to the political disturbances. The assistant research worker with the team could not be sent to Assam before May. He worked there till the end of June when the heavy rains prevented any further outdoor work from being carried out. The snails which were already exposed were brought down to Calcutta and were kept under observation. No *cercaria* have been found as yet.

A further supply of eggs from the worm in men and pigs and a batch of unexposed snails were also brought down. These snails have been exposed here in the laboratory and are still under observation. *Cyclops* were also exposed to *miracidia* with negative results.

The rainy season in Assam ends about the month of September and water begins to recede from the fields. The team proceeded to Assam in October to carry on the investigations.

19. Enquiry on allergic diseases and significance of endogenous allergens as regards ætiology, diagnosis and treatment of allergic diseases under the Director, Indian Institute for Medical Research, Calcutta.

The enquiry started functioning from the 1st July, 1947 and the work, which is being done, is of a preliminary nature. The following is an account of the researches in progress :—

Oriel and Barbar detected a nitrogenous matter, which they called proteose-like substance, in the urine of allergic cases. They claimed that this substance contains the specific allergen to which the patient is sensitive and may be effectively utilized in the treatment by method of hyposensitization. Truly allergic state is based on an antigen-antibody reaction; and making this his basis, Urbach classified the allergic conditions into two main groups, the exogenous and the endogenous allergy.

(a) *Exogenous allergy*—where the allergen is imported from outside the organism and exerts its effect through skin and mucous membrane, and it may be either :—

(i) Primary, exerting its effect as it is.

(ii) Secondary, becoming allergen only after being altered in the system.

(iii) Hapten or residual antigen being capable of reacting with specific antibodies but not producing them in the system.

(b) *Endogenous allergy*—where the allergen is elaborated in the system of the organism either by diseased tissues or bacterial matabolism. This type may be either :—

(i) Auto-endogenous, i.e. allergens manufactured by pervert tissues, they may be primary (directly from diseased tissues), secondary (from allergized tissues) or haptens (residual antigen).

or (ii) Hetero-endogenous source of allergens being bacterial metabolism in the system.

The p-substances were extracted from urine of allergic cases (presumably endogenous allergic cases, confirmed exogenous allergic cases and normal healthy individuals by Thier's modification of Oriel's original method (1930). Urinary proteoses extracted by Thier's method were tested for skin-tests and auto-proteose therapy.

(1) *Skin-tests.*— 0·1 c.c. of 1 : 1,000,000 dilution of standard solution (1 : 1,000) is injected in the anterior surface of fore-arm intra-cutaneously, if no reaction develops within 15 minutes, next lower dilution (1 : 100,000) is tried and so on. A control test is done with 0·1 c.c. of Evan's solution, the diluent of stock proteose solution. The signs indicating postive skin responses are :

(i) A definite blanched wheal,

(ii) Erythematous zone around the wheal with pseudopodia, and

(iii) These appearing within ½ hour to 1½ hour.

So far 28 cases of asthma were tested for skin-reaction with autogenous proteose substances, of which 20 cases showed immediate positive reactions. Certain number of cases complained of augmentation of symptoms. Two cases of urticaria were also tested with autogenous proteose substances; one responded immediately and the other after 2 hours, the latter complained excessive itching on the same night.

Specificity of skin-tests were not tested on this series of cases.

(2) *Therapeutic effect.*—Oriel's method was followed, e.g. to start with 0·1 c.c. of next higher dilution (10 times) of the solution giving positive skin-test injected subcutaneously. The subsequent doses were increased by 0·1 c.c. at weekly intervals.

Certain number of cases showed definite improvements, others did not respond to the treatment given to them.

Though our results were somewhat encouraging, negative effect in some well-established allergic conditions creates an anomalous position. Is it due to the fact that this p-substance is denaturalised during process of extraction by Thier's method which entails vigorous treatment of such a delicate substance with alcohol (95%) and subsequent drying in incubator ? According to Oriel, drying or even standing the substance for some time denaturalises it and this denaturalization may easily vitiate cutaneous reactivity and therapeutic effect with auto-proteose therapy. In fact, it has been observed that p-substances extracted by this method do not dissolve in Even's solution. To avoid this we have reverted to Oriel's modification (1933) to his own original method of extraction (1930). He changed his original method of extraction in the following ways :—

(i) Alcoholic precipitate after centrifugalization is washed in distilled water (7 c.c. to 8 c.c.) and re-centrifuged to eliminate various salts in alcoholic precipitates (e.g. urates), which are very apt to cause non-specific reaction owing to variable is otoxicity.

(ii) Final precipitate is readily dissolved in minimum amount of N/10 sodium hydroxide solution (0·5 c.c. to 1 c.c.) and the whole thing is diluted in Evan's solution as diluent to 10 c.c. This makes a stock of arbitrary dilution (1 : 1,000).

This method undoubtedly gives clearer proteose solution and positive skin-reactions with comparatively higher dilutions of stock solution. The p-substances extracted by Oriel's own modified method *vis-a-vis* Thier's method of extraction are being tested.

(3) At present the work is proceeding on the extraction of p-substances from 400 c.c. of urine of 24 hours' collection of urine passed during the active phase of the disease—both in endogenous and exogenous cases and also from 24 hours' collection of urine of healthy individuals. The p-substances obtained from 200 c.c. of urine are dissolved in N/10 Sodium Hydroxide solution and then in Evan's solution and are kept in frigidaire. From another 200 c.c. of urine p-substances are extracted, and dried in incubator to determine the quantitative alterations among the known endogenous, exogenous allergic cases as well as healthy normal individuals.

(4) From the dried samples, work is further proceeding to determine the more precise chemical structure of p-substances, both from exogenous and endogenous allergies. Oriel has shown that normal proteose differs from allergic samples in that it lacks the acetic acid and alcohol fractions of the latter, and even the trichloroacetic acid fraction of normal proteose does not give, unlike allergic p-substances, positive skin-reactions on the respective individual. But the p-substances from exogenous and endogenous cases have not been chemically studied, all of them being regarded as polypeptides composed of histidin, orginine, and lysine contaminated by nucleo-protein and clucosamine. If that be so that all alergic samples are of some chemical structure, how is it then that one differs so widely in skin reactivities and therapeutic effects from another ?

(5) *Urinary proteose—An endogenous allergen.*—Attempts are being made to perform complement-fixation tests using proteose solution as antigen.

(a) A rabbit was injected with a proteose-solution (1 : 1,000) intravenously :

(i) First injection 0·15 c.c. on 4-10-1947.

(ii) Second injection 0·5 c.c. on 8-10-1947.

(iii) Third injection 1·0 c.c. on 11-10-1947.

(iv) Fourth injection 1·5 c.c. on 15-10-1947.

(v) Fifth injection 2 c.c. on 27-10-1947.

(vi) Sixth injection 3 c.c. on 1-11-1947.

Animal was bled and the serum was tested for complement-fixing antibodies. A weak complement-fixation reaction was obtained.

(b) Samples of blood collected from 4 different allergic patients were tested for complement-fixing antibodies with the respective autogenous urinary proteose as antigens resulted in successful complement-fixation demonstrating presence of complement-fixing antibody. The method of extraction of urinary proteose and technique of complement-fixation test will be communicated later. These results have been obtained during October and November 1947 and are of fundamental importance in the Science of Allergy. Urbach was able to demonstrate the presence of complement-fixing antibodies in cases of hypersensitiveness to egg, milk and pollen, but always failed in cases of drug allergy. To our knowledge these results constitute first recorded observation of the presence of allergic antibodies in endogenous allergy and the significance of this method is obvious as it would enable us to forego experiments on animals and human beings in many instances.

20. Enquiry on the enteric group of fevers under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

This enquiry was begun in April 1947, and the work was directed mainly towards two problems :—

1. *A study of the relative incidence of the types belonging to the enteric group of fecers.*— This investigation was carried out in collaboration with the staff of the Medical College Hospital. So far 91 cases have been studied. The causative organisms were isolated in 29 cases (*Bact. typhosum* 27 and *Bact. paratyphosum* A2). In the remaining 62 the diagnosis was based on Widal reactions which included *Bact. typhosum* 54, *Bact. paratyphosum* A 5 and *Bact. paratyphosum* B 3 cases. Agglutination tests were carried out for *Bact. paratyphosum* C and *Bact. enteritides* in a few cases with negative results ; and further study of relative incidence is in progress now.

2. *A clinical study which included :—*

(a) *Variations in blood protein levels in the disease.*— Plasma proteins were estimated on 34 occasions in 13 patients, admitted into the hospital attached to the school. On admission, in these 13 patients, the mean concentration was found to be 6·0, 5·1 and 5·7 g., per 100 c.c. (compared with the normal figure of 7·1 g.) during the first, second and third weeks of illness, while the albumin/globulin fractions were 3·3/2·6, 2·8/2·2 and 2·9/2·8 g. per 100 c.c. Thus there was a definite fall in plasma proteins mainly due to reduction in the albumin fraction with little or no reduction of globulin. This is shown in the low 1·17/1 A/G ratio compared with a normal of 1·8/1 . There was history of marked restriction of diet with a low calorie intake of less than 1,000 before admission

in 5 cases, but low figure was also obtained in a number of cases with only moderate restriction of diet. In a few seriously ill patients the plasma protein rapidly fell down despite fairly good feeding while in the hospital. These observations indicate that the alteration in protein metabolism in typhoid leads to depletion of tissue proteins which is reflected in a drop of circulatory blood proteins, the albumin fraction in particular. Further observations showed a definite rise in total plasma protein and in the albumin fraction in all cases on discharge from the hospital, though not quite normal except in one. Plasma-protein estimations seem to have an important bearing on prognosis and treatment of enteric fever.

(b) *Absorption of glucose when given orally.*— The glucose-tolerance test was done in 15 cases. The fasting blood-sugar level rose from 80 mg. to 115 mg. per 100 c.c., giving a definitely high curve (diabetic type) in 9 cases, while sugar appeared in the urine in four. The test was repeated during convalescence in 2 cases showing a normal curve.

It is common to encounter typhoid patients admitted to the hospital in a semi-starved state with history of marked restriction in diet-protein in particular, while glucose is extensively used often parenterally in the treatment of enteric fever. The above observation clearly indicate the importance of adequate protein intake and show no advantages of parenteral administration of glucose ordinarily in this disease.

(c) *Diets* suitable for Indian patients in different phases of illness have been designed to maintain the general state of nutrition and to suit their appetite and taste. These diets about 2,500 calories are under trial at present. Diets with higher calories were attempted at first but we failed to induce patients to take them. We, however, feel that it is not essential to determine accurately the number of calories in the diet of each patient but he should be encouraged to eat as much as possible. In presence of abdominal distress or diarrhœa the diet is modified and reduced temporarily but often supplemented by intravenous glucose saline and/or plasma transfusion with extra vitamin supplements.

(d) *Treatment.*— Synergic action of penicillin and sulphathiazole was tried in six patients, 3 males and 3 females, aged 14, 9, 18, 29, 4 and 15 years, *Bact. typhosum* was isolated in 5 cases while the last one had only a positive Widal (TH 1-1,600). The duration of illness when treatment was started was 9, 11, 12, 17, 17 and 12 days. 4 patients were seriously ill one of whom was pregnant with anæmia and aborted soon after admission while the remaining two patients were non-toxic. The dosage of penicillin was 200,000 units 2 hourly in two and 3 hourly in one, 50,000 units 3 hourly in the child of 4 years, while the other two (age 14 and 9) had 30,000 and 10,000 units 3 hourly. Sulphathiazole injection was given in doses of 1 g., 3 or 4 hourly, preceded by an initial intravenous injection of 1 g. in 4 cases, the child of 4 having half the dose. Both drugs were given simultaneously for a period of 5 to 8 days. The range of temperature began to be lower and more fluctuating with institution of this treatment and the temperature became normal 6, 10, 7, 10 and 8 days respectively after the institution of this treatment; the last patient (bacteriologically negative) who was gravely ill died on the 3rd day of treatment. The marked toxaemia of the average cases appeared to be definitely reduced with this therapy. The number of cases so far treated is too small to warrant the drawing of any conclusion but the weight of evidence is that some cases at any rate are benefited by this treatment.

Further enquiry is proceeding.

List of papers published.

BRAGANCA, B. M., and RAO, M. V. RADHAKRISHNA.—Hypoprothrombinanæmia produced by Sulphathiazole in Rats on a Diet free of Vitamin K and cured by Synthetic Vitamin K (*I.J.M.R.*, 35, No. 1).

DAS GUPTA, C. R.—Value of Transfusion in the Treatment of Anæmia (*I.M.G.*, 82, No. 6).

DAS GUPTA, C. R., and CHATTERJEE, J. B.—Hypoproteinæmia due to Utilization of Plasma Protein for Erythropoiesis (*Ind. Med. Gaz.*).

DAY, C. D. MARSHALL.—Antiscorbutic Deficiency during Lactation as a Cause of Infantile Survey and Dental Dystrophies (*I.J.M.R.*, 35, No. 3).

DAY, C. D. MARSHALL, and SHOURIE, K. L.—The Effect of Pregnancy on the Incidence of Dental Caries in Indian Women (*I.J.M.R.*, 35, No. 2).

DAY, C. D. MARSHALL, and SHOURIE, K. L.—Hypertrophic Gingivitis in Indian Children and Adolescents (*I.J.M.R.*, 35, No. 4).

DAY, C. D. MARSHALL, and SHOURIE, K. L.—Attrition and Temporomandibular Joint Changes in Relation to Dental Prosthesis in India (*in press*).

GHOSH, L. M., DEY, N. C., and PANJA, D.—Studies on Ringworm. Part IV. Incidence of *Trichophytom gypseum* in India (*Ind. Med. Gaz.*, 82, No. 2, p. 73).

PANJA, D., DEY, N. C., and GHOSH, L. M.—*Sporotrichosis* of the Skin in India (A new species described) (*Ind. Med. Gaz.*, 82, No. 4, p. 200).

SHOURIE, K. L.—A Dental Survey in Ajmer-Merwara (*I.J.M.R.*, 35, No. 3).

REPORT OF THE FIFTH MEETING OF THE CLINICAL RESEARCH ADVISORY COMMITTEE HELD IN NEW DELHI ON THE 9th DECEMBER, 1947.

MEMBERS PRESENT.

Dr. V. R. Khanolkar. (*Chairman*).
Dr. R. N. Chaudhuri.
Dr. C. R. Das Gupta.
Dr. A. N. Goyle.
Dr. S. W. Hardikar.
R. B. Dr. M. G. Kini.
Dr. P. Kutumbiah.
Lieut.-Colonel S. Narain.
Dr. J. C. Ray.
Dr. B. B. Yodh.
Dr. J. C. Patel. (*Secretary*).

Dr. Jivraj N. Mehta, Chairman of the Scientific Advisory Board of the Indian Research Fund Association kindly attended the meeting.

Dr. R. N. Cooper, Honorary Surgeon, K. E. M. Hospital, Bombay was not able to attend the meeting being out of India.

BY INVITATION.

Dr. S. Banerjee.
Dr. A. C. Chatterjee.
Dr. R. E. Dadachanji.
Dr. Dharmendra.
Dr. P. V. George.
Dr. P. V. Gharpure.
Lieut.-Colonel S. D. S. Greval.
Lieut.-Colonel B. N. Hajra.
Dr. J. Jhirad.
Dr. C. O. Karunakaran.
Dr. M. N. Lahiri.
Lieut.-Colonel C. K. Lakshmanan.
Dr. A. R. Mehta.
Dr. T. Bhaskara Menon.
Dr. V. R. Naidu.
Dr. H. Patil.
Major N. G. S. Raghavan.
Dr. D. N. Roy.
Dr. D. L. Shrivastava.
Dr. D. W. Soman.

The Chairman in his introductory remarks welcomed the new members Dr. R. N. Chaudhuri, Dr. C. R. Das Gupta, Lieut.-Colonel S. Narain and Dr. J. C. Ray. He stressed the need of curtailing the expenditure on the new and old enquiries due to the present financial stringency. He hoped that better times would dawn by the beginning of 1949-50.

It was suggested at the meeting that workers who apply for grants-in-aid for enquiries under the Indian Research Fund Association should be invited

to attend the meetings of the Advisory Committees in order that they might be able to speak on their own enquiries.

Dr. Jivraj N. Mehta in reply drew attention to the fact that this point was considered by the Clinical Research Advisory Committee at its 4th meeting held on the 10th December, 1946 and the Scientific Advisory Board in December 1946, and instructions had been sent out that persons who submitted applications for grant should contact workers from their localities who were likely to attend the meetings of the Advisory Committees and explain their problems to them so as to enable them to sponsor their proposals. He added that there would be no objection to persons attending the meetings of the Advisory Committees at their own expense. The Committee, therefore, did not consider it necessary to alter its former recommendation.

Item 1.—The minutes of the 4th meeting of the Clinical Research Advisory Committee held in the Haffkine Institute, Bombay, on the 10th December, 1946, were adopted.

Item 2.—The Committee then proceeded to consider the progress of work on enquiries sanctioned during the year 1947-48 :—

(i) Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay.

The Committee approved the work carried out by the unit and recommended its continuation. It was further recommended that Dr. V. S. Waravdekar should be provided in the grade for research officers, viz. Rs. 350-350-380-30-590-E.B.-30-770-40-850.

(ii) Histopathological unit at the Tata Memorial Hospital, Bombay.

The need for the urgent establishment of a neuropathological unit was stressed and the Committee recommended that the I.R.F.A. should favourably consider the early establishment of such a unit.

(iii) Enquiry on Naga sore under Dr. S. R. Pandit, Director, Pasteur Institute, Shillong.

The continuation of this enquiry for 9 months during 1948-49 was recommended with revised budget not exceeding Rs. 7,000.

(iv) Enquiry into medical mycology under Dr. L. M. Ghosh at the School of Tropical Medicine, Calcutta.

The continuation of this enquiry was not recommended.

(v) It was noted that the enquiry into the infections of the feet under R. B. Dr. M. G. Kini at the Stanley Hospital, Madras, would terminate at the end of this financial year.

(vi) Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay.

The continuation of this enquiry for one year only was recommended. At the end of the year Dr. M. V. Radhakrishna Rao may be requested to review the progress of the work as it would be the end of the 5th year of its existence.

(vii) Enquiry on cirrhosis in animals and its bearing on the ætiology of infantile biliary cirrhosis under Dr. T. Bhaskara Menon at the Andhra Medical College, Vizagapatam.

The continuation of this enquiry was recommended for one year only.

(viii) Enquiry on infantile cirrhosis under Dr. P. Kutumbiah at the Stanley Medical College, Madras.

It was noted that this enquiry had terminated.

(ix) Enquiry entitled clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Professors V. Subrahmanyan and V. R. Naidu at the Indian Institute of Science, Bangalore and Sri Krishnarajendra Hospital, Mysore.

The continuation of this enquiry was recommended.

(x) Dental research unit under Dr. C. D. Marshall Day at the deMontmorency College of Dentistry, Lahore.

It was noted that this enquiry had terminated.

(xi) Enquiry into the relationship between the measurements of vertebræ and body size of Indians under Dr. R. G. Ginde at the Seth G. S. Medical College, Bombay.

It was noted that work on this enquiry had terminated due to Dr. Ginde being selected as a Government of India scholar for advanced study abroad.

(xii) Enquiry into causes of blindness under Major E. J. Somerset at the Medical College, Calcutta.

It was noted that this enquiry would terminate on the 31st March, 1948.

(xiii) Enquiry on typhus under Dr. D. W. Soman based at the Haffkine Institute, Bombay.

The continuation of this enquiry was recommended for one year only with a revised budget of Rs. 13,500.

(xiv) Enquiry into typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta.

The continuation of this enquiry was recommended for one year only with a revised budget of Rs. 14,000.

(xv) Enquiry on the effect of vitamins C and B in diabetes under Dr. S. Banerji at the School of Tropical Medicine, Calcutta.

The continuation of this enquiry was recommended on low priority with a revised budget of Rs. 10,000.

(xvi) Research on lathyrism in the Central Provinces & Berar.

The report of the Lathyrism Sub-Committee was adopted. (*Appendix I*).

(xvii) Survey of hookworm diseases in the Jharia Coalfields Settlement Area under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

It was noted that work on this enquiry would be completed by the 31st March, 1948.

(xviii) Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

The continuation of this unit was recommended for one year with a revised budget of Rs. 10,535.

(xix) Enquiry on kala-azar under Dr. P. C. Sen Gupta at the School of Tropical Medicine, Calcutta.

The continuation of this enquiry was not recommended.

(xx) Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

The continuation of this enquiry was recommended for one year with a rider that it should terminate at the end of 1948-49.

(xxi) Enquiry to investigate allergic diseases and significance of endogenous allergens as regards etiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

The continuation of this enquiry was recommended for one year only with a revised budget of Rs. 8,520.

(xxii) Enquiry on enteric group of fevers under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta.

The continuation of this enquiry was recommended for one year only with a revised budget of Rs. 5,452.

Item 3.—To consider new proposals.

(A) The Committee made the following recommendations in regard to new enquiries.

(i) Enquiry to investigate bactericidal, protistocidal and fungicidal action of phytoncides under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

This enquiry was recommended for one year only.

(ii) Enquiry on the neurogenic factor in the production of traumatic shock and its treatment by the alteration of cerebro-spinal fluid electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay.

This enquiry was recommended for one year only with a high priority.

(iii) Enquiry into erythroblastosis fœtalis and the Rh factor under Lieut.-Colonel S. D. S. Greval, Imperial Serologist to the Government of India, School of Tropical Medicine, Calcutta.

This enquiry was recommended for one year only with a high priority.

(iv) Enquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay.

This enquiry was recommended for one year with a low priority.

(B) The following new enquiries were not recommended in view of the financial conditions mentioned above :—

(i) Enquiry on the state of refraction of Indian children of different communities with special reference to myopia under Dr. G. S. Pendse, 631/27, Sadashiv Peth, Poona 2.

(ii) Enquiry on the properties of an anti-biotic agent from a polyporus fungus under Professor S. R. Bose, Professor of Botany, Carmichael Medical College, Calcutta.

(iii) Enquiry on summer gastroenteritis in the United Provinces under Dr. A. Mukherji, Medical Officer, Provincial Hygiene Institute, Lucknow.

(iv) Enquiry on the pathogenicity and antigenic relationship of lactose-non-fermenting and feeble lactose-fermenting organisms other than the definitely known pathogens in stools of normal and diseased population of India under Dr. G. Panja, Professor of Bacteriology and Pathology, School of Tropical Medicine, Calcutta.

(v) Enquiry on bone marrow under Dr. D. Govinda Reddy, Professor of Pathology, Medical College, Madras.

(vi) Enquiry on alloxan diabetes under Dr. S. Banerjee, Diabetes Research Worker, School of Tropical Medicine, Calcutta.

(vii) Enquiry on complement-fixation reaction in gonorrhœa under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

(viii) Enquiry on leukemia in relation to some enzymes and vitamins under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

(ix) Enquiry into isolation of intestinal pathogens and classification of salmonellas under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta.

(x) Enquiry into the coccidial infection of man under Dr. S. Sen, officiating Professor of Protozoology, School of Tropical Medicine, Calcutta.

(xi) Enquiry into the inheritance of blood groups under Lieut.-Colonel S. D. S. Greval, Imperial Serologist, School of Tropical Medicine, Calcutta.

(xii) Enquiry on venereal diseases in Calcutta under Lieut.-Colonel S. D. S. Greval, Imperial Serologist, School of Tropical Medicine, Calcutta.

(xiii) Investigation into the chromatophrotropic hormone of the pituitary gland and its employment in the treatment of pigmentary disorders of the skin, e.g. leucodarma, vitiligo, etc. under Dr. B. Mukerji, Director, Central Drugs Laboratory, Calcutta.

(xiv) Enquiry into the role of protein nutrition in the production, recurrence and healing of peptic ulcer under Dr. P. Raghavan, Honorary Physician, K. E. M. Hospital and Seth G. S. Medical College and Dr. R. G. Chitre, Assistant Professor of Biochemistry, Seth G. S. Medical College, Bombay.

Item 4.—To consider extracts from the minutes of the 6th and 7th meetings of the Medical Research Liaison Committee for India held in December 1946 and March 1947, respectively regarding infantile biliary cirrhosis.

Recorded.

Item 5.—To consider extracts from the minutes of the 6th, 7th and 8th meetings of the Medical Research Liaison Committee for India held in December 1946, March 1947 and June 1947 respectively regarding lathyrism.

Recorded.

Item 6.—To note section 2 (I) (d) of the minutes of the 7th meeting of the Medical Research Liaison Committee for India held in March 1947 regarding medical mycology.

Recorded.

Item 7.—To consider section 4 of the minutes of the 8th meeting of the Medical Research Liaison Committee for India held in June 1947 regarding sprue.

Recorded.

Item 8.—Organization for clinical trials of new remedies under the Indian Research Fund Association.

A Sub-Committee consisting of Dr. P. Kutumbiah, R. B. Dr. M. G. Kini, Dr. B. B. Yodh, Dr. C. R. Das Gupta and Dr. J. C. Patel was appointed to examine this question. The report of the Sub-Committee is appended as *Appendix II* to this *Report*.

The following items of the agenda which read as under were deferred for consideration while awaiting the adoption by the Indian Research Fund Association of the recommendation embodied in item 8 above :—

(i) To note extracts from the minutes of the 6th and 7th meetings of the Medical Research Liaison Committee for India held in December 1946 and March 1947 respectively regarding trials with V187 in the treatment of tetanus.

(ii) To consider a letter from the Oriental Pharmaceutical Industries Ltd., Bombay regarding carrying out of clinical trials of "Asthmopil".

(iii) To consider the request from Messrs. Boots Pure Drug (India) Ltd., Bombay that clinical trails of "Flavazole" and "Sulphathiazole" be carried out.

APPENDIX I.

Report of the meeting of the Lathyrism Sub-Committee held in New Delhi on the 8th December, 1947.

The Sub-Committee was glad to learn that the liability with regard to the financial responsibility for the personnel required for field and clinical investigations has now been accepted by the Government of Central Provinces & Berar and that the Government have sanctioned the necessary budget for the prosecution of the enquiry for three years. In view of this the Committee recommended that Major Bhave, Dr. Khanolkar and Dr. Patwardhan may be asked to prepare a budget for the year 1948-49 not exceeding Rs. 17,000 for the I.R.F.A. portion of the enquiry.

The Sub-Committee recommended that the therapeutic trials suggested by Major Bhave may be carried out if that work does not interfere with the plan which was accepted by the Clinical Research Advisory Committee in December 1946.

The Sub-Committee realise the problem is also of importance to the Province of Bihar. They recommend that the authorities in Bihar should keep in close contact with the work on the present scheme and also in regard to the therapeutic trials.

The Sub-Committee recorded its thanks to the Secretary, Medical Research Liaison Committee for India, London, for placing at its disposal a number of reprints of Spanish papers on lathyrism. These papers will remain with the Secretary, I.R.F.A. and can be consulted by the workers interested in lathyrism.

The Committee feels the extreme need of a neurohistologist for studies in lathyrism and other similar problems and recommends that early steps may be taken to recruit a suitably qualified person for the work.

APPENDIX II.

Report of the Sub-Committee appointed by the Clinical Research Advisory Committee to report on item No. 7 of the Agenda regarding the setting up of an organisation for the conduct of trials of new remedies under the Indian Research Fund Association.

The Sub-Committee met at 9 a.m. on the 10th December, 1947 in Committee Room 'B', North Block, Imperial Secretariat, New Delhi.

PRESENT.

Dr. P. Kutumbiah. (*Chairman*).
R. B. Dr. M. G. Kini.
Dr. B. B. Yodh.
Dr. C. R. Das Gupta.
Dr. J. C. Patel. (*Secretary*).

Dr. P. Kutumbiah was asked to take the Chair.

The Sub-Committee made the following recommendations :—

(i) that the Indian Research Fund Association should undertake controlled clinical trials of suitable new drugs, appliances and apparatuses.

(ii) that a Sub-Committee of the Clinical Research Advisory Committee called the Therapeutic Trials Sub-Committee be formed which should consist of :—

(a) One physician.
(b) One surgeon.
(c) One pharmacologist.
Of these one should be a member of the Clinical Research Advisory Committee.
(d) A representative of the Scientific Advisory Board.

(iii) that the Sub-Committee be empowered to co-opt members if the drug is for any special group of diseases such as diseases of the skin, the eye, etc.

This Sub-Committee will obtain the necessary information regarding facilities available in different institutions in India for this type of work through the office of the I.R.F.A.

This Sub-Committee will in the first instance decide the suitability of the drug for the trial.

This Sub-Committee will then collect data regarding the suitability of the place and personnel who should be invited to undertake the trial.

It is anticipated that the work of this Sub-Committee will be carried out by correspondence and personal contact will be maintained when found necessary.

The Sub-Committee will meet once a year before the annual meeting of the Clinical Research Advisory Committee and submit its report to the Committee.

The Sub-Committee will, in consultation with those entrusted in the carrying out of clinical trials, recommend the general lines on which the trial will be conducted.

The Sub-Committee will recommend the requirements for individual trials, viz. personnel and financial commitments.

The report of the work done will be submitted to the I.R.F.A. through the Therapeutic Trials Sub-Comimttee.

MATERNITY AND CHILD WELFARE.

1. Enquiry into the bearing of premature and immature births on infant mortality under Dr. B. C. Das Gupta, Executive Health Officer, Bombay Municipality.

The investigation which commenced on the 1st September, 1946, was undertaken with the object of studying the bearing of premature and immature births on the incidence of infant mortality.

Data for investigation was obtained from thirteen dependable institutions in the city, where it was found that complete records would be available. These institutions cater for all classes and communities, and hence a fairly representative sample was obtained for the investigation.

Owing to the impossibility of obtaining a pathologist, the inquiry is not quite as complete as desired; however, the larger institutions and some of the smaller ones, were able to supply some pathological data which proved valuable in the investigation.

Scope of the work.— For the purposes of the enquiry, any viable infant weighing upto 4 lb. 15 oz. was considered premature or immature, according to the period of pregnancy as obtained from a careful study of the pre-natal and natal case forms, and the history obtained from the mother; from the physical characterstics of the infants, such as the weight, length and circumference of the head, the colour of the skin, the adipose tissue, the cry and the vitality of the infant.

The report is an interim one preceding the final report, which will be presented in January, and the figures against "Information yet to be ascertained" in this report will as far as possible be collected, and entered in the final report.

The total number of mothers who gave birth to premature and immature infants was 2,817, and the total number of such infants was 2,922; the 105 extra infants were—101 of twins and 4 of triplets.

Infant findings.— 1,282 of 43·9% of the infants were premature and 1,476 or 50·5% were immature. Information is yet to be ascertained in 164 or 5·6% of the cases.

Sex.—There were 1,519 or 52% female infants and 1,897 or 47·8% males; in 6 or 0·2% of the cases, information is yet to be ascertained.

It will be noticed that female infants exceeded the males by 4·2%.

Period of maturity :—

TABLE I.

Period.	Total No.	Percentage.	No. of infant deaths.	Percentage.
28th week	363	12·4	243	8·4
32nd week	584	20·0	165	5·6
36th week	335	11·5	35	1·2
40th week	1,476	50·5	158	5·6
Information to be ascertained ..	164	5·6	40	1·2
Total ..	2,922	100·0	641	22·0

Of the total number of premature infants, the largest number had a maturity of 32 weeks, and of those that expired, the largest number were at the 28th week of maturity.

Grouping of infants by weight :—

TABLE II.

Weight.	Total Infants.	Percentage.	No. of Infant deaths.	Percentage.
(i) 1 lb.-2 oz. to 2 lb. ..	87	3·0	78	2·7
(ii) 2 lb.-1 oz. to 3 lb. ..	317	10·9	188	6·4
(iii) 3 lb.-1 oz. to 3 lb.-8 oz. ..	287	9·83	107	3·7
(iv) 3 lb.-9 oz. to 4 lb. ..	518	17·7	102	3·6
(v) 4 lb.-1 oz. to 4 lb.-15 oz. ..	1,711	58·5	164	5·6
Information yet to be ascertained	2	0·07	2	0·07
Total ..	2,922	100·0	641	22·07

Ninety% of premature infants fell in the first 3 groups, and 10% in the 4th group.

Ninety % of the immatures fell in the 5th group and 10% in the 4th group.

Among those infants who succumbed, there were the largest number of deaths in the 2nd group, and the maturity of these infants were from the 28th to the 32nd week.

Average birth weights in the different periods of maturity tabulated as under :—

TABLE III.

28th week	2 lb.-4 oz.
32nd week	3 lb.
36th week	4 lb.-4 oz.
40th week	4 lb.-10 oz.

(5) The average length of infants, the circumference of the head, and the weight of the placentas at different period of maturity is tabulated as under :—

TABLE IV.

Averages.	28th week.	32nd week.	36th week.	40th week.
Length	17″	17½″	17½″	18½″
Circumference of head ..	11¾″	12″	12½″	13″
Weight of the placentae ..	12 oz.	14 oz.	1 lb.	1 lb.

(6) *Details of infant mortality.*—Six hundred and forty-one or 22% of the infants expired, 327 or 11·2% of those were males, and 313 or 10·7% were females, information of the sex of one is still to be ascertained. The deaths among male infants was higher than females by 0·5%.

Four hundred and forty-three or 69·1% of the infant deaths were among premature infants, and 158 or 24·6% among immature infants. Information in the case of 40 or 6·3% is still to be ascertained.

Five hundred and nine or 17·5% of the infants expired in institutions, and 132 or 4·5% after discharge; 52 or 1·7% of the latter succumbed in the neo-natal period, and 77 or 2·6% after the neo-natal period. The period of death in 3 cases is still to be ascertained.

Period of infant deaths is tabulted as under :—

TABLE V.

Period.	Number.	Percentage.	
1st week	509	17·5	Neo-natal deaths 19·2%
2nd to 4th week ..	52	1·7	
After 1st month ..	77	2·6	
Information yet to be ascertained ..	3	0·2	

Thus neo-natal deaths so far investigated formed 19·2% of the total infants.

Of the total infants under study, 641 died, of which 56 were twins or one of twins and 55 were infants of mothers whose parity ranged from 7th to 16th para. Each group formed about 1·9% of the total infants.

(7) The causes of infant deaths in the different periods of maturity is grouped in Table VI in *Appendix A*.

Maternal findings.—The total number of mothers under enquiry was 2,817. Multiple pregnancies numbered 184 or 4·7% of the total births.

(I) *Parity.*—

Nine hundred and ninety-two or 35·2% of the mothers were prima-paras and 1,820 or 64·6% were multi-paras, and 5 or 0·2% have yet to be ascertained. Two hundred and twenty-one or 7·8% of the multi-paras were mothers whose parity ranged from a 7th to a 16th para which was the highest parity among these mothers.

In 609 or 21·5% of the mothers the infants succumbed, and parity among these mothers was prima-para 223 or 7·9% and multi-paras 350 or 12·4%. The parity in 36 or 1·2% is yet to be ascertained.

(II) *Age-groups of mothers tabulated as under.*—

TABLE VII.

Ages.	Total No.	Percentage.	Mothers whose infants expired.	Percentage.	Percentage based on total infant mortality.
Below 20 ..	460	16·3	109	3·9	17·9
Between 20-30 ..	2,009	71·3	421	14·9	69·1
„ 31-35 ..	237	8·4	43	1·5	7·1
„ 36-40 ..	80	2·9	24	0·8	3·9
Above 40 ..	8	0·3	4	0·1	0·7
Information yet to be ascertained ..	23	0·8	8	0·3	1·3
Total ..	2,817	100·0	609	21·5	100.0

It will be noticed that the largest number of mothers who bore underweight infants were in the 20 to 30 age group. This is not unusual, for it is found that in mothers who gave birth to full term mature infants, the largest number occur in this age group, which is considered the most fertile period for mothers.

A comparison of ages between both parents showed a disparity in 4 or 0·1% of the cases.

(III) *Distribution of mothers by communities tabulated as under.—*

TABLE VIII.

Community	Total No.	Percentage.	Mothers whose infants expired.	Percentage.
Hindus	2,064	73·30	436	15·5
Mohammedans	397	14·10	88	3·1
Indian Christians	3·3	10·40	77	2·7
Jews	49	1·70	7	0·2
Parsees	12	0·42	1	0·03
Anglo-Indians	1	0·04	..	.. .
Information yet to be ascertained	1	0·04	..	
Total	2,817	100·0	609	21·53

The very large number of Hindu mothers can be accounted for by the fact that Hindus form two-thirds of the total population of the city, but apart from this fact, most of the contributory causes leading to premature and immature births are more common among them, particularly among those under investigation. The chief factor in a large majority being the very inadequate and unbalanced diet consumed. Unlike the other communities, a large percentage of Hindu mothers in this study had a lower economic status and were forced to earn their living to supplement their husbands' earning. The chief occupation followed by them is that of work in textile mills besides other minor occupations followed by a few. In such instances, they carried on their work to an advanced period of pregnancy. In addition, they had more strenuous domestic duties, and their standard of living was necessarily low. Again, although the Indian Christians are one-third the number of the total population of Mohammedans, the number of premature births among them was comparatively high. The reason amongst other contributory factors was, that in addition to a low economic status, their diets were extremely poor, lacking all the essentials of a normal balanced diet.

(IV) *History of previous pregnancies and labours.—*

Those pregnancies and labours which were normal and not complicated by any disease or untoward events, and where all the children were living, have been termed good. Those where the state of the mothers' health was not quite normal and where a history of a few infant deaths accompanied by some trouble during labour occurred were styled fair. Whereas cases in which previous pregnancies showed a series of still-births, abortions, premature births and early

infant deaths, together with complications during labour have been included in the category ' bad ' tabulated as under :—

TABLE IX.

	Total No.	Percentage.	Mothers whose infants expired.	Percentage.
Good	608	21·6	106	3·7
Fair	509	18·1	90	3·2
Bad	569	20·2	154	5·4
Information yet to be ascertained	139	4·9	36	1·3
Prima-paras	992	35·2	223	7·9
Total ..	2,817	100·0	609	21·5

(V) *Pre-natal care.*—

The majority of mothers were under pre-natal care tabulated as under :—

TABLE X.

	Total No.	Percentage.	Mothers whose infants expired.	Percentage.
Under pre-natal care ..	2,146	76·2	419	14·8
No care	524	18·6	159	5·6
Information yet to be ascertained	147	5·2	31	1·1
Total	2,817	100·0	609	21·5

Of those mothers under pre-natal care, the average number of visits to clinics was 3 per mother. In addition they registered at the clinic in the last two months of pregnancy. At the clinics, only clinical examinations were done and at the most certain medicines provided. Arrangements for other amenities as extra nourishment, such as milk or meals have not been provided. Besides, the follow-up-work of the mothers in their houses by the health visitors to see if instructions given at the clinics were being carried out was inadequate.

(VI) *Health history of present pregnancies and labours.*—

The health of the mothers was satisfactory in a comparatively small number of cases (b48). The pregnancies of the remaining were complicated in some instances by more than one disease, which accounts for the disparity in numbers between the total mothers and the pathological conditions and other factors present, which contributed towards bringing about a premature or immature delivery.

Only severe anæmias with a hæmoglobin percentage ranging from 25% to 55% and which were mostly secondary, (mal-nutrition contributing in a large measure to these) formed 50% of diseases present. Acute diseases occurred

in 19·8% of the mothers. Chronic diseases were present in 1·9%. Complications of pregnancy and labour in 13·9% and such untoward events as falls, journeys, with their consequent exertion and jostling, over-work and the lifting of heavy weights, and emotional causes like fright, anxiety, and grief together caused 10·1% of the total conditions present (Table XI) :—

TABLE XI.

Diseases.	Total No.	Percentage.	Mothers whose infants expired.	Percentage.
Severe anæmias	1,428	50·0	364	12·9
Toxæmia	210	7·4	136	4·8
Eclampsia	23	0·8	9	0·3
Versions	10	0·8	10	0·3
Ante-partum hæmorrhage (separation of placenta) ..	123	4·3	48	1·7
Hydramnios	30	1·06	13	0·4
Osteomalscia (causing disproportion)	5	0·1	3	0·1
Syphilis (Wasserman positive) ..	31	1·1	13	0·4
Diarrhœa	177	6·2	40	1·4
Dysentery	78	2·7	15	0·5
Pneumonia	12	0·4	4	0·1
Asthamatic attacks	24	0·8	4	0·1
Heart disease	22	0·7	6	0·2
Tuberculosis	34	1·2	16	0·5
Influenza	218	7·7	43	1·5
Fever with rigors	54	1·9	30	1·0
Pyrexia	64	2·2	10	0·3
Measles	1	0·03	1	0·03
Smallpox	1	0·03	1	0·03
Typhoid	1	0·03	1	0·03
Typhus	2	0·07	..	..
Ankylostomaisis	17	0·6	3	0·1
Falls causing troma ..	172	6·1	85	1·3
Journeys	65	2·3	18	0·6
Lifting of heavy weights ..	31	1·1	10	0·3
Emotional	17	0·6	7	0·2
Information yet to be ascertained	189	6·7	..	..

(VII) *Pathological examinations* so far obtained show the following results :—

	Total No.	Results. Positive.	Results. Negative.	Results in mothers whose infants expired. Positive.
(i) Wasserman or Kahn ..	176	31	145	13
(ii) Blood counts in severe anæmias	120	Shows (C.I.) & /or B.C. much below normal.	..	8
(iii) Microscopic examination of stools for ova, cists, amœba	105	79	26	3
ankylostoma	..	6	90	3
(iv) Smears for malaria parasites	18	3	15	3
(v) Sputum for T.B. bacili ..	2	2	..	1
(vi) Widal for typhoid ..	1	..	1	..
(vii) Van den Berg for bile salts ..	1	1	..	..
(viii) *X*-rays for tubercular lungs	4	4	..	4

Although blood counts were done in 120 cases of severe anæmia, they are far short of the total number of severe anæmia cases shown in Table XI. The extra number styled as fever anæmia was obtained from the clinical records maintained at the institutions and where no counts were available.

(VIII) *Period of pregnancy at which labour occurred.*—

TABLE XII.

Period.	Total Mothers.	Percentage.	Mothers whose infants expired.	Percentage.	
7th month ..	329	11·7	231	8·2	Premature
8th month ..	565	20·1	156	5·5	Premature
9th (beginning) ..	830	11·1	25	1·2	Premature
Full term ..	1,439	51·6	156	5·5	Immature
Information yet to be ascertained ..	154	5·5	31	1·1	
Total ..	2,817	100·0	609	21·5	

Thus 51·6% or a little over half the total mothers gave birth to full term but immature infants, which shows that in the sample under enquiry premature and immature births occurred in about the same proportion. A reference to Table I shows that 15·2% of the infant deaths occurred in premature infants and 5·6% in immature infants, a proportion of 3 to 1, which goes to prove that in the number under investigation, the immature infants stood a better chance

of survival and that prematurity and the period of gestation have a greater bearing on the incidence of infant mortality than immaturity.

(X) *Nature of present labours* (Table XIII) :—

TABLE XIII.

	Total Mothers.	Percentage.	Mothers whose infants expired.	Percentage.
Normal	2,571	91·3	552	19·5
Abnormal	78	2·7	11	0·4
Forceps	36	1·3	8	0·3
Ceasarean section ..	18	0·6	7	0·2
Other artificial interference ..	39	1·4	15	0·5
Information yet to be ascertained	75	2·7	16	0·5
Total ..	2,817	100·0	609	21·5

The abnormal labours were those in which some mal-presentations occurred. The instrumental labours were forceps deliveries. Operative were Caesarean section. Artificial interference included medical induction, artificial rupture of membranes, episiotomy and versions.

Post-partus hæmorrhage occurred in 60 or 2·1% of the mothers.

(XI) *Puerperium* (Table XIV) :—

TABLE XIV.

	Total mothers.	Percentage.	Mothers whose infants died.	Percentage.
Febrile	394	13·9	121	4·3
Afebrile	1,683	59·7	323	11·4
Information yet to be ascertained	740	26·4	165	5·8
Total ..	2,817	100·0	609	21·5

(XII) *Maternal mortality.*—

Thirty-seven of the total mothers or 1·3% expired and the causes of death are tabulated as under :—

TABLE XV.

	Total No.	Percentage.	Mothers whose infants expired.	Percentage.
Sepsis	9	0·32	3	0·1
Manual removal of placenta and post-partum hæmorrhage ..	1	0·03	..	..
Cardiac failure	3	0·1	1	0·3
T.B. lungs	3	0·1	3	0·1
Severe anæmias	7	0·21	5	0·2
Diarrhœa and fever	2	0·07	2	0·07
Eclamptic fits	1	0·03	1	0·03
Typhoid	1	0·03	..	..
Toxæmia	2	0·07	..	..
Abdominal disease	1	0·03	1	0·03
Information yet to be ascertained	7	0·21	2	0·07

(XIII) *Other information.*—

Of the 2,817 mothers, 10 or 0·35% were unmarried, 7 or 0·24% were deserted by their husbands, and 24 or 0·85% were widows.

(XIV) *Social status* (Table XVI) :—

TABLE XVI.

	Total No.	Percentage.	Mothers whose infants expired.	Percentage.
Good	62	2·2	21	0·7
Fair	512	18·2	81	2·9
Bad	1,744	61·9	476	16·8
Information to be ascertained	499	17·7	32	1·1
Total ..	2,817	100·0	609	21·5

(XV) *Occupation of both parents* (Table XVII) :—

TABLE XVII.

Occupation.	Fathers.	Percentage.	Mothers.	Percentage.	Mothers whose infants expired.	Percentage.	Percentage on the basis of infant mortality.
Service	1,104	39·1	62	2·2	16	0·6	2·6
Mill hands	541	19·2	81	2·9	17	0·6	2·8
Petty trades	358	12·7	10	0·4	10	0·4	1·7
Mechanics	213	7·6					
Shopkeepers	65	2·3					
Businessmen	44	1·6					
Coolies	85	3·1	13	0·4			
Sweepers	84	3·0	53	1·9	13	0·5	2·1
Professions	20	0·7	6	0·2			
Bidi workers	..	..	17	0·6	9	0·3	1·5
Unemployed	72	2·5	2,575	91·4	544	19·1	89·3
Information to be ascertained	231	8·2					
Total ..	2,817	100·0	2,817	100·0	609	21·5	100·0

(XVI) *Income of families :—*

TABLE XVIII.

	Total families.	Percentage.	Families whose infants expired.	Percentage.
Up to Rs. 60	1,672	59·3	392	13·9
From Rs. 61 to Rs. 150 ..	542	19·2	149	5·3
Rs. 151 and upwards ..	32	1·1	22	0·8
Unemployed	72	2·6	14	0·4
Information yet to be ascertained	499	17·8	32	1·1
Total ..	2,817	100·0	609	21·5

From Tables XVI, XVII and XVIII, it will be observed that scarcely 2% of the families had a good social status with an income of Rs. 150 and above. Nineteen % of the families had just enough to be able to live within their means, and are described as fair, while 62% had an income ranging from *nil* to Rs. 60. Their social status was consequently bad, with an income barely sufficient for their daily needs. It is in this group that the largest number of premature and immature births and infant deaths (in 13·9% of the 21·5 % deaths) so far ascertained occurred.

(XVII) *Home visits :—*

In order to ascertain the standard of living, housing conditions, diets, state of nutrition and health of the inmates in the house, 1,400 or 50% of the mothers have so far been visited. Repeated visits at periodical intervals have also been paid in order to check the progress or otherwise of the infants. 6·5% of the mothers visited, could not be traced in the homes, as they had either given an incorrect address in the institution at the time of admission, or shifted subsequently. Efforts are now being made to trace them.

(XVIII) *Standard of living :—*

TABLE XIX.

	Total families.	Percentage.	Families whose infants expired.	Percentage.
(*A*) *Standard of living.*				
Good	41	1·4	11	0·3
Fair	135	4·8	33	1·1
Bad	1,032	36·9	328	11·7
Information to be ascertained ..	192	6·9	237*	8·4
Total ..	1,400	50·0	609	21·5

	Total families.	Percentage.	Families whose infants expired.	Percentage.
(B) Housing.				
Good	40	1·4	11	0·3
Fair	162	5·8	41	1·4
Bad	1,006	25·9	320	11·4
Information to be ascertained ..	192	6·9	237*	8·4
Total ..	1,400	50·0	609	21·5
(C) Diets.				
Good	39	1·4	11	0·3
Fair	113	4·0	41	1·4
Bad	1,056	37·7	320	11·4
Not traced ..	192	6·9	..	..
Information to be ascertained ..	..	..	237*	8·4
Total ..	1,400	50·0	609	21·5

* With reference to 237 cases, the families of these 237 infants who expired in the institutions have not so far been visited in their homes. However, barring the standard of living, diets and housing conditions, the rest of the information about these is available in the previous portion of the *Report*.

Vegetarians.—55% or 1·9% of the total mothers visited were vegetarians. Of these 11 or 1·8% lost their infants.

Extra milk taken.—Only 99 or 3·5% of the mothers visited took milk as an extra article of diet and among these in 26 or 2·3%, the infants succumbed.

It will be observed that the figures in Table XIX(A), (B) and (C) more or less correspond. Thus they are on an average 1·4% good, 4·7% fair, and 37% bad. This is a natural result of the larger percentage of families where the economic status is low, and the income meagre, tending towards causing a low standard of living and housing and diets which are inadequate and unbalanced.

Comparing these figures with those in which infant deaths occurred, it will be seen that the same conditions hold. For, here as well, the figures correspond, being on an average 0·3% good, 1·1% fair, and 11·5% bad.

Housing.—The living conditions in 25% of mothers were deplorable and left much to be desired. They resided in single rooms which were overcrowded and ill-ventilated. Washing facilities were insufficient or even absent. Very insanitary and unhealthy conditions existed in the surroundings of 10% of the dwellings.

Diets :—The average caloric value of the diets works out to C 1,500 per person per day, which is far below the average requirements of an individual. They lacked in protective foods, proteins, fats and vitamins, and were unbalanced and inadequate to satisfy or sustain the mothers, who were in consequence mal-nourished and under-fed.

This very poor state of social conditions took its toll on the mothers' health and having no reserve energy or stamina, she was unable to bear the

additional burden and strain of pregnancy, accompanied as it was by malnutrition. This produced a very poor state of health in the mothers as is shown by a high anæmia index which finally resulted in the birth of premature and immature infants whose vitality was such that they were incapable of survival.

(XIX) *Causes.*— From the information so far ascertained, prematurity and immaturity can be ascribed to the following causes in the mother :—

(1) Multiple pregnancy.
(2) Repeated pregnancy.
(3) Hydramanios.
(4) Versions (converted breech).
(5) Anti-partum hæmorrhage (separation of the placenta).
(6) Induction of premature labour.
(7) Fæcal mal-formation.
(8) Syphilis.
(9) Toxæmias.
(10) Severe anæmias.
(11) Acute diseases.
(12) Chronic diseases.
(13) Overwork.
(14) Trauma following falls, journeys, etc.
(15) Emotional.
(16) Unknown.

Conclusions.—No definite conclusions can be arrived at until all the mothers have been visited and full information obtained. However, of the 50% mothers who have so far been followed at their homes, certain inferences can be drawn from facts revealed as follows :—

(1) That immature births were larger in number than premature births, i.e. 50·5% to 43·8%.
(2) That deaths in premature infants were far higher than immature, i.e. 15·2% to 5·6% and that most of these deaths occurred in the 28th week of maturity.
(3) That neo-natal deaths, and chiefly those in the first week of life were high, i.e. 19·2% of the total 22·0% deaths so far ascertained.
(4) That of the premature infants the largest number were in the 32nd week of maturity.
(5) That the largest number of births in mothers occurred in the 25-30 age group.
(6) (a) That 70% of the total mothers were under pre-natal care.
(b) That total visits by mothers to the clinic averaged only 3 per mother, showing that ante-natal care was ultimately inadequate and in most instances belated.
(c) That of the 21·5% mothers whose infants succumbed, 14·6% were under pre-natal care.

(d) That registration at the clinic took place at a very late period of pregnancy.

(7) That on the whole the health of the mothers was not satisfactory and of the pathological conditions present, severe anæmias formed 50% of the total conditions.

(8) That in a large percentage of the mothers, the social conditions, such as economic status, standard of living, housing and diets were bad and largely responsible for causing the premature and immature births which occurred in the sample under investigation.

(9) That pathological examinations were comparatively few, and in several instances, facilities for such did not exist. Hence diagnosis and treatment which could have combated or prevented many of the conditions was started when the disease was far advanced and was, therefore, of little benefit.

APPENDIX A.

TABLE VI.

Causes.	28th week.	Percentage.	32nd week.	Percentage.	36th week.	Percentage.	40th week.	Percentage.	Unknown.	Percentage.	Total Causes.	Percentage.
Congenital debility	226	7·8	109	3·7	15	0·51	54	1·85	23	0·8	427	14·66
Asphyxia ..	6	0·2	8	0·27	3	0·1	13	0·44	..	..	30	1·01
Toxicity arising from toxæmia on mothers	3	0·1	5	0·17	1	0·03	3	0·1	..	..	12	0·4
Congenital syphilis	2	0·07	5	0·17	1	0·03	2	0·07	..	..	10	0·34
Hæmorrhages ..	2	0·07	2	0·07	..	..	1	0·03	..	..	5	0·17
Pyrexia ..	3	0·1	7	0·24	3	0·1	15	0·51	2	0·07	30	1·02
Marasmus ..	1	0·03	6	0·2	5	0·17	21	0·72	..	..	33	1·12
Atelectasis	..	..	4	0·13	..	..	..	..	..	..	4	0·13
Crænial injury	..	..	1	0·03	1	0·03	5	0.17	..	..	7	0·3
Umbilical sepsis	..	..	1	0·03	..	..	..	..	..	..	1	0·03
Jaundice ..	..	..	2	0·07	..	..	4	0·13	6	..	6	0·21
Convulsions	..	..	4	0·13	1	0·03	6	0·2	3	0·1	14	0·5
Congenital malformation	..	..	2	0·07	..	..	2	0·07	..	..	4	0·14
Diarrhœa ..	..	..	3	0·1	1	0·03	10	0·34	..	..	14	0·5
Thrush	..	..	..	..	1	0·03	1	0·03	..	..	2	0·07
Pneumonia	..	..	..	..	2	0·07	2	0·07	..	..	4	0·14
Monsters	..	..	..	..	..	..	3	0·01	..	..	3	0·01
Abscess	..	..	..	..	..	..	..		3	0·1	3	0·01
Unknown	..	..	6	0.2	1	0·08	16	0·04	9	0·3	32	1·0
Total. Total percentage to total infant deaths	243	8·37 8·4	165	5·58 5·6	35	1·18 1·2	153	5·37 5·4	40	1·37 1·4	641	22·0
Percentage to total mothers under enquiry		37·9		25·7		5·5		24·7		6·2		10·0

2. Enquiry on anæmias in pregnancy under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay.

Since the beginning of enquiry in June 1947, 21 cases have so far been taken up for study. Each case was thoroughly investigated according to the scheme and a detailed record was maintained of history, physical examination and complete laboratory investigations done. The following were findings of the cases. Out of the 21 cases, 19 were of anæmia associated with pregnancy while 2 were clinically normal cases. Out of these one case of anæmia could not be completely investigated as the patient left hospital against advice before the investigations were complete.

1. Total red blood cell count in millions/c.mm.

0—1	2
1—2	7
2—3	6
3—4	3
4—5	2
Total	20

2. Hæmoglobin in grammes per 100·0 c.c. of blood

2—3	3
3—4	1
4—5	6
5—6	6
6—7	1
8—9	1
9—10	1
11—12	1
Total	20

3. Cell volume in c.c. per 100 c.c. of blood ..

5—10	1
10—15	7
15—20	6
20—25	2
25—30	2
30—35	1
35—40	1
Total	20

4. M.C.V. above 94·0 c.μ 8
78·0 7
less than 78·0 5
Total 20

5MCH above 320γ γ 4
270-32γ γ 5
less than 270 γ γ 11
Total 20

6. MCHC—32-38% 9
less than 32% 11
Total 20

7. Sya MCD by P. J. curve

6–7μ	3
7–8μ	12
8–9μ	1
Total	16

8. Van den Bergh reaction and icterus index showed hyper-bilirubinæmia in 5 cases.

9. Gastric analysis giving alcohol meal and injection of histamine acid phosphate if necessary—2 cases of histamine refractory achlorydria.

10. Marrowgram.
    Normoblastic reaction— 8
    Megaloblastic reaction—12
11. Plasma proteins—less than 5 g./100 c.c. in 5 cases.
12. Blood N.P.N. and blood cholsterol—normal values.
13. Serological—Kahn test + + in 4 cases.
14. Ankylestoma ova detected in 2 cases.
15. Urine urobilinogen was within normal range in all cases.
16. Bleeding and coagulative time—normal in all cases.
17. E.S.R.—increased in 6 out of 13 cases in which it was done.
18. Fragility of rbc—increased in 2 cases, diminished in 1 and normal in rest.

All the patients belonged to the working class in Bombay and were from 7th to 9th month pregnant at the time of admission. The common symptoms were weakness, breathlessness, œdema, loss of appetite, diarrhœa, sore-mouth and tongue, and fever. A detailed record of their dietetic history, hygienic condition, economic status, etc. is being maintained.

The number of cases is too small to draw any conclusion. The work done so far shows the existence of different types of anæmias associated with the pregnancy state. It is needless to state that the treatment of these cases would depend on the type of anæmia exhibited by the pregnant woman.

3. Enquiry into the reproductive patterns of urban and rural population under Dr. C. Chandra Sekar, Professor of Statistics and Dr. Muktha Sen, Professor of Maternity & Child Welfare at the All-India Institute of Hygiene & Public Health, Calcutta.

Two field staff Mrs. U. Das Gupta and Mrs. B. Bhattacharjee and a peon were appointed towards the end of June. A certain amount of preparation of the field was necessary before the actual work started. The objects of the enquiry and the method of conducting it were given publicity in local papers and some propaganda was made in the field, to get the support of the public. The staff were also given preliminary training for three weeks to get them acquainted with the general principles of survey work.

The areas selected for the work are : (1) a locality in central Calcutta served by Sir John Anderson Health School clinic and inhabited mostly by middle class Bengali Hindu families, and (2) a few villages in Singur Health Centre area. Considering the season of the year in which the work was started, it was decided to work first in Calcutta and later in Singur. The field work in Calcutta is continuing and at the end of the year both the staff will be shifted to Singur to carry on the enquiry there.

Up to the end of October 1947, 800 individual schedules have been completed. The work was to a certain extent adversely affected by the occasional communal disturbances in the city.

Some analysis of the 532 *schedules are given below.*—The age group selected were, all the women between the ages of 12 and 50 years.

Marital status.

Married and with husband.	Widows.	Separated.	Unmarried.	Total.
337	54	7	134	532

Province of origin.

Bengal.	U.P.	Bihar.	Total.
537	3	2	542

Average age at marriage 13-9.

Family limitation was attempted by 41 and 18 desired but did not.

Knowledge of contraception.

Satisfactory.	Unsatisfactory.	*Nil.*
37	16	268

Total births were 1,237 of these live 1,135, still 22, and abortions 80.

Number of terminated pregnancies according to the age of the mother.

Age in years.	12-14,	15-19,	20-24,	25-29,	30-34,	35-39,	40-44,	45-50.
Number of pregnancies.	0'2	0'6	1'6	3'1	4'1	4'5	4'6	7'9

Average number of pregnancies per woman—3·1.

The few analysis done till now is promising interesting information.

REPORT OF THE MATERNITY AND CHILD WELFARE ADVISORY COMMITTEE.

The Committee met at 10.00 a.m. on the 10th December, 1947 in the Imperial Secretariat, North Block, New Delhi.

PRESENT.

Dr. J. Jhirad. (*Chairman*).
Dr. A. Benjamin.
Miss M. Craig.
Dr. C. R. Das Gupta.
Dr. J. M. Orkney.
Dr. N. A. Purandare.
Rao Bahadur Dr. K. C. K. E. Raja.
Dr. Muktha Sen.
Dr. S. Pandit. (*Secretary*).

BY INVITATION.

Major-General A. C. Chatterji.
Dr. B. C. Das Gupta.
Lieut.-Col. C. K. Lakshmanan.
Dr. (Miss) H. Patil.

1. The Committee considered the progress reports of the following enquiries sanctioned during the year 1947-48 :—

(i) *Enquiry into anæmia in pregnancy in the Singur Health Unit under Dr. Muktha Sen, at the All-India Institute of Hygiene & Public Health, Calcutta.*

Dr. M. Sen informed the Committee that, as workers were not available, the enquiry could not be started and that she did not propose to take up the work during 1948-49. The Committee, therefore, recommended that the enquiry be dropped for the present.

(ii) *Enquiry into bearing of premature and immature births on infant mortality in Bombay under Dr. B. C. Das Gupta.*

Dr. Das Gupta informed the Committee that the collection of fresh data ceased on 31st July, 1947. Information yet to be ascertained from institutions and from home visits was being completed and he expected that, on the completion of the final report by the end of January, 1948, the staff engaged would be disbanded.

The preliminary report submitted by him was discussed by the members and a few suggestions were made particularly with regard to presentation of statistical tables.

The Chairman thanked Dr. Das Gupta for having undertaken this enquiry, and for the valuable information he had so far collected. She deplored the fact that the services of a pathologist were not available to throw light on some of the obscure problems in this field.

In discussing the classification of premature and immature births the Committee recalled the work done by Dr. Ghosh on 'Standards of Maturity

of Indian Infants' which was recommended for publication at the meeting of the Maternity and Child Welfare Advisory Committee held in Bombay on 10th December 1947.

Dr. Raja moved that, for future enquiries of this nature, the Committee should appoint a small Sub-Committee to be associated with the person in charge of the enquiry for drawing up a schedule on which the required information will be collected. The institutions from which information is to be secured should be contacted before the enquiry is started and their co-operation should be obtained in order to ensure that the data collected are on the lines which are desired.

(iii) *Enquiry into the pelvic type and its relationship to body-build in Indian women under Dr. S. K. Basu, Dr. S. Mitra and Dr. S. Mukerjee, at the Carmichael Medical College, Calcutta.*

It was reported that the enquiry was not undertaken for want of use of an *x*-ray plant but that they hoped that it will be possible to take up the work in 1948-49. The Committee recommended its being taken up next year but felt that in view of the difficulty of an *x*-ray plant, this enquiry might be given a lower priority than others.

(iv) *Enquiry on the anæmia of pregnancy, under Dr. R. G. Dhayagude, at the Seth G. S. Medical College, Bombay.*

The Committee noted that, so far, only a small number of cases had been investigated but expected valuable information from this investigation and recommended its continuation during 1948-49.

(v) *Enquiry into the reproductive patterns of urban and rural population under Drs. C. Chandra Sekar and Muktha Sen, at the All-India Institute of Hygiene & Public Health, Calcutta.*

Dr. Muktha Sen reported on the work done so far and was hopeful of collecting interesting data if the enquiry was continued and extended. She pleaded for extra workers. The Committee, however, felt that it should be possible for them to carry out the proposed extended enquiry with the staff already engaged and recommended only the addition of a computer. The Committee recommended that the enquiry should end with the financial year 1948-49.

2. Proposals for New Enquiries.

Enquiry on anæmia in pregnancy under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta.

Dr. C. R. Das Gupta explained his scheme of investigation in the rural area of Kaurapurkar, six miles from Calcutta. The proposal was discussed fully. In view of the anticipated difficulty in getting a sufficient number of subjects for repeated follow-up and in view of the prevalence of hookworm infection and malaria in the population as complicating factors promoting anæmia, it was felt that it would not be justifiable to recommend the scheme as put up by Dr. Das Gupta. The Committee recommended, however, that a pilot enquiry be carried out during 1948-49, with a limited staff consisting of a technician, a woman assistant and a peon and considered that a decision regarding the fuller investigation should be based on the results made available to the Committee from the pilot enquiry.

3. The Committee considered the revised draft report of the summary of findings of investigations into the causes of maternal mortality in India, prepared by Dr. S. Pandit, and approved it with very slight modifications. Dr. Raja proposed a vote of thanks to Dr. Pandit for compiling the summary and this was seconded by Dr. Purandare. The Committee recommended its publication and circulation to provincial and municipal health departments.

4. The Chairman stated that, following on the enquiry by Dr. B. C. Das Gupta on the bearing of premature and immature births on infant mortality, a more extensive enquiry into infant deaths was indicated. Discussing the advisability of such an enquiry the Committee recommended that a Sub-Committee consisting of Dr. Raja, Dr. B. C. Das Gupta, Dr. Orkney, Dr. Muktha Sen, Dr. Benjamin and Dr. S. Pandit (Secretary), be formed to draw up a memorandum on the conduct of enquiries into infantile mortality.

5. The Committee also recommended that wider publicity should be given to the subjects for research as suggested vide minute 8 of the meeting held in November 1945.

FILARIASIS.

1. Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.

The progress of work of this enquiry was considerably hampered on account of the disturances which were prevalent in the city. It was not possible to collect blood at night and the only source of materials was the out-patients' clinic which was also very poorly attended, and then suitable cases were very few. It is only very recently that the situation is coming back to normal and it is expected that materials in adequate quantities will be available again for the experimental work.

Screening tests.— *In vitro* experiments were performed with several drugs on the microfilariæ collected from venous blood and hydrocele fluid of men, after the method advocated by Hawking. As the research staff could not come for night work in the laboratory owing to continued curfew orders in the city, the hospital staff collected samples of blood in citrate saline at night and the experiments were performed the next morning. The microfilariæ obtained in this way were quite active and healthy-looking and the experiments were put up with these as scheduled and a parallel set of experiments was also done with the microfilariæ collected from the same patients in the day time. This was necessary to rule out the debilitating action, if any, of the citrate saline on the microfilariæ kept overnight. Experiments were also made on the laking of blood for concentrating the microfilariæ. By the method advocated by Hawking, it took some time for the blood to be completely laked; it has been found that by use of saponin obtained from bazar soap-nuts, laking was complete in a shorter period thus allowing more time for the screening experiments.

The drugs used for screening tests were made into solution of different strengths from 1 in 10 up to 1 in 50,000 or more. Preliminary observations were made at room temperature by putting in living microfilariæ in small amounts of the different solution of the drugs on slides, which were kept in a moist chamber in between the observations. Examinations were made at intervals of 5, 10, 15, 30, 45 minutes and 1 hour and a rough idea of the microfilaricidal action of the drug was first formed. Subsequently, experiments were put up with the microfilariæ in different solutions of the drugs in small test tubes (after Hawking) and left overnight at 37° C. These were examined the next morning (after 19 hours). It was found that the minimum lethal concentration after 19 hours were: stibatin, 1 : 1,000, anthiomaline, 1 : 200, paludrine, 1 : 32,000, miracil, 1 : 50,000, R-301, 1 : 500.

These are the five drugs tested so far. The rest of the drugs in hand will be tested during the remaining part of the current year and the next year.

Intradermal test for diagnosis of filariasis.—As stated before the disburbed conditions made the collection of living *Dirofilaria immitis* from dogs very difficult. The incidence of this worm in pariah dogs in Calcutta is very poor (practically *nil*) as reported by Maplestone and Bhaduri (*I. J.M.R.*, 1940). Only one specimen was obtained through the kindness of the Principal, Bengal Veterinary College. The situation coming back to normal, it is expected that more specimens will be obtained through him and other sources.

The antigen prepared from the single worm was used on several patients but the skin-reactions were not satisfactory.

Field work.—There was a plan to set up a field station at a suitable endemic area near Calcutta to study the methods of control of filariasis. But owing to the disturbed conditions and lack of an experienced assistant research worker, the work could not be started. It is hoped that next year an experienced man will be available for the field work and a suitable place in Bengal will be fixed up.

2. Enquiry to evaluate the intradermal and other immunological tests in the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras.

The enquiry was primarily sanctioned for the purpose of instituting laboratory procedures for the early diagnosis of filariasis, especially by the intradermal tests with the filarid antigens. The Medical Officer sanctioned for the enquiry, joined duty on the 18th June, 1947. After preliminary training and review of current literature, the work of preparation of antigens was undertaken from the 1st of August, 1947. Work at present is being restricted to the preparation of suitable antigens from the filarid, *Conispiculum guindiensis*, the parasite of the garden lizards, *Calotes Versicolar*. Four hundred and fifty-six lizards were examined, out of which 169, or 37·6 %, were found to be infected. From the filarid worms so obtained, two types of antigens were prepared :—

(a) Acetone extract antigen.

(b) Saline extract antigen.

2. It was also decided to prepare an antigen from the infective larvæ as they occur in the probosois of the mosquito, *Culex fatigans.* The larval stages of this filarid had been described previously by Pamdit *et al.* in *C. fatigans.* Number of batches of mosquitoes were raised, fed on infected lizards and after a suitable period of development, antigen was prepared from the infective larvæ. After a sufficient quantity of this antigen is available, comparative tests with all the three antigens so far prepared will be undertaken.

3. During the course of this work, it was noted that some of the lizards had shown an apparently elephantoid condition in one or both of their limbs. This was a new finding. This condition is under experimental investigation and the report on the histological changes in the affected limbs will be submitted to the Conference in due course. While Bhaskara Menon and his collaborators have reported the production of a similar condition by experimental procedures, it is believed that this will be the first instance of it occurring in nature in any animal.

3. Enquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona and Dr. K. Venkatraman, Director of the Technology Department of the University of Bombay.

Synergistic action of drugs and wetting agents has been found to potentiate the value of drugs. However, a study of the action of surface active chemotherapeuticals, i.e. compounds modified in their chemical constitution by the introduction of structural features which lead to surface activity, does not seem to have received proper attention. It would appear that drugs

endowed with wetting power would be more effective therapeutic agents, especially against organisms which are resistant to attack by virtue of their peculiar physical character. In this connection, study of surface active chemotherapeuticals for the treatment of filariasis was deemed interesting.

The existing filarial therapy almost borders on empiricism and little systematic work on the subject is on record. However, using 'hit or miss' tactics numerous drugs with well-marked therapeutic value in allied protozoal diseases have been tested *in vitro* and *in vivo* for their efficacy in different filarid infections. None of these have proved the "magic bullet" for the disease. To some extent antimonials, especially trivalent ones, have been found to be beneficial in the treatment of the disease. Very recently, cyanine dyes have been reported as effective chemotherapeutic agents in filariasis.

The present enquiry was started about the middle of June and synthesis of surface active derivatives of compounds with probable value in filariasis have been undertaken.

Salts of methylene blue, which is known to possess selective staining power and bacteriostatic value and of astraphloxine FF, a typical cyanine dye, with wetting agents containing a sodium sulphonate or sulphato group, such as Nekal BX (sodium disopropyl naphthalene sulphonate) and Gardinol WA (sodium lauryl sulphate) have been prepared. In order to standardize conditions for the preparation of salts of these compounds with wetting agents, their salts with sodium naphthalene β-sulphonate, were first synthesized. All these compounds are new and await pharmacological investigation. The salts are water-soluble, but to a much less extent than their components. They were obtained as amorphous, sticky products which could not be crystallized from the usual organic solvents. They have, however, been obtained in analytically pure form by chromatographic absorption.

Starting from gallic acid and potassium antimony tartrate, antimony subgallate has been obtained and the compound has been condensed *in situ* with *n*-dodecylamine in the presence of pyridine and phosphorus trichloride to the alkylamide. It is insoluble in water and is being further studied. An alternative route to this substance through the corresponding triacetyl gallamide and introduction of antimony in the final step is also being explored.

Stibanilic acid has been prepared, but owing to the difficulties experienced, in common with other workers, poor yield of the compound has been obtained. The amine has been diazotised and coupled with hexylresorcinol to form a new azo dye.

REPORT OF THE MEETING OF THE FILARIASIS ADVISORY COMMITTEE HELD ON THE 8th DECEMBER, 1947.

The Committee met at 2-30 p.m. on the 8th December, 1947.

MEMBERS PRESENT.

Dr. C. G. Pandit. *(Chairman).*
Dr. R. G. Dhayagude.
Dr. B. B. Dikshit.
Lieut.-Colonel Jaswant Singh.
Dr. A. K. Mukerji.
Dr. M. O. T. Iyengar. *(Secretary).*

IN ATTENDANCE.

Dr. A. S. Arora.
Major A. N. Duggal.
Dr. J. C. Gupta.
Lieut.-Colonel B. N. Hajra.
Dr. P. Kumaran Nair.
Dr. D. R. Mehta.
Dr. S. R. Pandit.
Dr. I. M. Puri.
Major N. G. S. Raghavan.
Dr. T. Ramachandra Rao.
Dr. D. N. Roy.
Dr. N. Veeraraghavan.
Dr. K. V. Venkataraman.
Dr. D. K. Vishwanathan.
Dr. K. S. Viswanathan.

1. The Committee considered the work carried out by the three enquiries sanctioned during 1947-48.

(a) *Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta.*

Dr. Mukerji gave an account of the work done under this enquiry during the current year, and stated that as no suitable worker was available for dealing with field work on filariasis, namely field surveys and filariasis control work, this aspect of the enquiry could not be carried out. As there was no possibility of recruiting a suitable worker in the near future, Dr. Mukerji agreed, on the suggestion of the Committee, to modify the proposal for 1948-49 so as to restrict the scope of the enquiry to (1) screening work and (2) intradermal tests for the diagnosis of filariasis, and to exclude field work.

Dr. Mukerji then proposed the following revised estimate for 1948-49:—

One Research Assistant on Rs. 170/- p.m.	..	Rs. 2,040
One Laboratory Assistant on Rs. 87/- p.m.	..	Rs. 1,044
One Laboratory Assistant on Rs. 60/- p.m.	..	Rs. 720
One Sweeper on Rs. 30/- p.m.	..	Rs. 360
Dearness allowance to above staff	..	Rs. 1,680
Compensatory and house rent allowances	..	Rs. 1,300
Contingencies and chemicals	..	Rs. 500
	Total	Rs. 7,644

The revised proposal was accepted unanimously and recommended by the Committee*.

(b) *Enquiry to test the usefulness of the intradermal and other immunological tests in the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras.*

Dr. C. G. Pandit gave an account of the work done during the current year by this enquiry, and outlined the work planned for 1948-49. The Committee unanimously recommended the grant of Rs. 7,008 for the continuance of the enquiry during 1948-49.

(c) *Enquiry on changing the physical properties of drugs by "Wetting Agents" under Dr. B. B. Dikshit and Dr. K. V. Venkataraman.*

Dr. Dikshit gave an account of the work done by this enquiry during the current year and outlined the future plan of work. The Committee unanimously recommended the grant of Rs. 9,957 for the continuance of this enquiry during 1948-49.

In regard to the three enquiries recommended by the Committee the following priority was recommended:—

(1) Enquiry under Dr. B. B. Dikshit and Dr. K. V. Venkataraman.

(2) (a) Filariasis enquiry under Dr. A. K. Mukerji.

(b) Enquiry on intradermal and other immunological tests under the Director, King Institute, Guindy, Madras.

2. The Committee considered and noted the extracts from the minutes of the 6th, 7th and 8th meetings of the Medical Research Liaison Committee for India held in December 1946, March 1947 and June 1947, respectively, regarding investigation of new drugs according to the methods suggested by Dr. F. Hawking for screening test on microfilariæ.

3. The Committee considered and recorded a note entitled "Experimental Filariasis of Cotton Rats—Investigations at the National Institute for Medical Research" by F. Hawking and A. Burroughs.

4. Dr. M. O. T. Iyengar spoke on the need for a central organization for the investigation of the epidemiology of filariasis in India, endemicity of filariasis in different parts of the country, distribution of the two species of filariæ parasitic in man and determination of the vector species of mosquitoes responsible for transmission of the infection in the different localities. He stated that although in regard to malaria investigations, survey and control, there exist organizations in all provinces and a big organization at the Centre, no such organizations exist in regard to the study of control of filariasis. He felt that it may not be feasible for the provinces at this stage to have any special staff for this purpose, and suggested that there should be a central organization either under the I.R.F.A. or under the Government of India. Such an organization would serve to carry out local investigations, help the Provincial Governments in the formulation of schemes for filariasis control, stimulate their interest in such schemes and direct and supervise the schemes if desired. He moved the following resolution:—

"This Committee recommends to the Scientific Advisory Board the constitution of a Central Organization expressly for the study of the epidemiology of filariasis in India with a view to (i) carry out investigations to

* The original proposal was for Rs. 22,102/-.

determine the distribution and endemicity of filariasis in different parts of India; (ii) determine the filarial species involved and the vector species of mosquitoes responsible for the transmission of filarial infection in different endemic areas; and (iii) formulate schemes for filariasis control and demonstrate the technique of control measures, thus stimulating the inauguration of filariasis control measures by Provincial Governments and local bodies.

Such a control unit specifically meant for this purpose, may, if desired, be attached to the Malaria Institute of India".

The resolution was seconded by Dr. B. B. Dikshit and passed unanimously by the Committee.

INDUSTRIAL HEALTH.

1. Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta.

The object of the unit is to form a nucleus team of workers specialized in the different aspects of industrial hygiene. This team is expected not only to have an all-round knowledge of the theory and practice of industrial hygiene, but would be in a position to undertake fundamental investigations. In addition, this unit will collaborate with research workers in universities or elsewhere and in the words of the Chairman, Industrial Health Advisory Committee, " The workers trained by this unit would blaze the trail of industrial research in the universities."

Due to difficulties in obtaining proper personnel full complement of the unit has not yet been obtained. Only three of the five officers have so far joined. The other two are expected to join shortly. In view of the fact that the Committee has recommended during its third meeting that " This group of workers will no doubt have to be trained in their respective fields before they can undertake research ", the officers are having their special training in the three months' course being conducted by the All-India Institute of Hygiene & Public Health from October 1947. By the end of December 1947, the course will be finished and the officers would be in a position to devote their whole time to research work from January 1948.

Meanwhile, the work on the following three lines is just started :—

1. *The incidence of lead-poisoning in printing presses.*

Two Government-owned presses in Calcutta have been chosen for the purpose of the investigation and the lines of work are as follows :—

(a) Industrial air analysis with the use of Midget impinger and, if possible, with electrostatic precipitator.

(b) Clinical and hæmatological examination of a representative sample of workers.

(c) Estimation of lead in urine and blood to assess sub-clinical lead-poisoning.

2. *The relation of environmental warmth to comfort.*

The object is to gauge the different factors of environmental warmth encountered in Indian industries that determine the physical comfort. Fourteen different environmental factors are being studied since 1st October, 1947 on a 24-hour basis twice a week. These values are being collected with a view to correlate the comfort number on a 7-point scale recorded by 12 volunteers.

3. *Effects of excess noise on the workers.*

The object is to study organic damage done to the jute workers by being exposed to the severe noise for eight hours a day. A local jute factory (Adamjee Jute Mills) has kindly consented for the investigation to be carried on in their premises. It is, therefore, proposed to commence the work as from the 1st November. One hundred workers with more than 10 years continuous experience in the jute industries, will be examined for their auditory acuity with the help of tuning forks and audiometer. To make the work complete the effects of noise on the efficiency of workers with a view to elicit the role of noise in fatigue will be carried out. General radio noise level meter would be used to conduct the

preliminary noise survey of different jute industries to select the group of experimental subjects exposed to maximum noise.

2. Enquiry into environmental conditions in certain industries in and around Calcutta under Prof. K. Subrahmanyan at the All-India Institute of Hygiene & Public Health, Calcutta.

The season has been cloudy and rainy with high humidity and reduced sunlight.

The survey is being conducted in two large printing presses in Calcutta and in two large jute mills outside Calcutta. The conditions observed are temperature (Globe thermometer, dry bulb and wet bulb), air movement (by Kata thermometer and anæmometer), light and daylight factor, dust, and sanitary facilities. There are 11 sampling points in one jute mill and 24 in the other.

Most of the departments in the presses have artificial lighting, but the presses themselves are not located in buildings designed for the purpose. Consequently, there is a certain amount of congestion of traffic in alloys.

The walls in all four factories are of brick but the roofs are terraced in the two presses and corrugated iron in the jute mills.

There are no women workers in the presses but 20% of the labour in the jute mills consist of women. All four factories have a continuous piped supply of safe drinking water, but the latrines are inadequate and not very well maintained.

Dust counts at the batching, preparing, and spinning departments in both jute mills are " innumerable ".

The observations should be continued for at least a complete year and some of the observations should be made so as to get an idea of the variation in conditions during the day in summer and winter. Data on output and turnover of labour will also be helpful.

A summary of median observations gathered so far is given in the Table below :—

TABLE.

ENVIRONMENTAL CONDITIONS IN FACTORIES.

Median values of observations taken during the period 15-7-1947 *to* 15-10-1947.

No. 1 *Press.*

Department	Dry bulb °F.	Humidity %	Air movement f.p.m.	Light f.c.
Linotype ..	86·5	76·5	134	6
,, ..	87·5	75·0	177·5	2
Ludlow type ..	88·5	73·0	79·0	14
Composing ..	85·0	79·5	160·0	1
Reading ..	85·5	79·5	91·5	1
Casting ..	92·0	70·5	45·0	1
Melting ..	89·0	76·5	63·5	10
Printing ..	86·5	78·0	66·5	1
External condition ..	87·0	80·0	110·0	202

TABLE (*Concld.*).

Department	Dry bulb °F.	Humidity %	Air movement f.p.m.	Light f.c.
	No. 2 Press.			
Reading ..	88·0	75·0	175·0	3
Monotype ..	90·0	68·0	65·0	18
Handpress ..	89·0	75·0	31·0	0
Composing ..	88·0	75·0	120·0	2
Linotype ..	90·0	68·0	100·0	15
Monoperforation ..	89·0	72·0	125·0	3
Binding ..	88·0	68·0	40·0	1
Envelope making ..	87·0	80·0	47·0	1
Counting ..	86·0	79·5	73·5	0
Printing ..	87·0	77·5	24·0	0
Printing ..	88·0	78·0	35·0	1
Workshop ..	85·0	82·0	47·0	1
External condition ..	87·0	81·0	62·0	200
	No. 1 Jute Mills.			
Batching ..	88·5	78·0	26·5	5·5
Batching ..	88·0	78·0	42·5	5·75
Spinning ..	90·0	76·5	68·0	7·5
Spinning ..	89·5	75·0	87·5	6·0
Winding ..	89·5	75·0	36·0	7·0
Beaming ..	93·0	70·5	38·5	4·0
Weaving ..	90·0	75·0	40·0	6·0
Finishing ..	90·5	76·5	45·5	2·5
General ..	88·0	78·0	40·0	3·0
External condition ..	88·0	78·0	390·0	440·0
	No. 2 Jute Mills.			
Batching ..	88·0	78·0	39·0	10
Batching ..	89·0	81·0	36·0	11
Batching ..	87·0	81·0	50·0	10
Preparing ..	89·0	75·0	45·0	5
Preparing ..	88·0	78·0	55·0	8
Preparing ..	88·0	75·0	45·0	9
Preparing ..	88·0	78·0	43·0	5
Spinning ..	90·0	75·0	41·0	5
Spinning ..	90·0	72·0	48·0	4
Spinning ..	91·0	71·0	26·0	6
Spinning ..	91·0	75·0	42·0	5
Finishing ..	90·0	75·0	30·0	6
Finishing ..	91·0	70·0	35·0	4
Finishing ..	91·0	72·0	30·0	4
Finishing ..	93·0	69·0	22·0	3·5
Beaming and weaving ..	94·0	68·0	30·0	3
Beaming and weaving ..	91·0	72·0	56·0	5
Beaming and weaving ..	93·0	71·0	45·0	4
Beaming and weaving ..	91·0	72·0	46·0	4
Winding ..	90·0	75·0	30·0	8
Winding ..	90·0	75·0	40·0	5
Winding ..	89·0	78·0	30·0	11
Winding ..	90·0	76·0	50·0	5
External condition ..	87·0	67·0	210·0	500

3. Enquiry on fatigue and efficiency in paper and textile industries under Professor Kali Prasad at the Laboratories of Experimental Psychology, Lucknow University, Lucknow.

Maximum output is contingent on maximum fitness of the individual worker and the object of the enquiry is to explore the sources of fatigue and to determine the most suitable conditions of work and efficiency.

After the appointment of the staff, the enquiry commenced sometime towards the end of June and the work was started in Cawnpore because it is an important textile centre and the means of conveyance and transport are easily available. Out of number of mills that were visited, the Swadeshi Mills was selected as the venue of the investigation. The Swadeshi is the most up-to-date of all the mills and has 82,732 spindles, 1,740 looms and 9,404 workers.

A preliminary fact-finding survey was made through personal observations and the factory records. A random sample of the factory's population was then taken which embraced all departments and mill-sections and included all strata of workers with due weight to their age and experience.

The measure adopted is output mainly because in our present state of knowledge, output is probably the most reliable index of fitness on the part of the workers.

But an early collection of statistical information was handicapped on account of the continuous disturbances in Cawnpore. Within a short period of three or four months since the commencement of the enquiry the staff has worked hard but has been compelled to take the help of the records maintained in the factory, knowing full well that they may not be adequate for a precise determination of the fatigue phenomenon. For instance, some figures of all the shifts of a sample of spinning section have been taken and subjected to statistical analysis. The data are, however, crude and require to be supplemented by more detailed information.

The statistical analysis of the collected figures show certain peculiarities which can be classified as follows :—

A. (1) The second days of the weeks record marked diminution in output.

(2) The seventh days show a general rise as compared to the sixth.

(3) The fourth days in most cases are marked with a rise and in other a level. Only in a few cases, there is a decline on that day.

B. The data when plotted graphically are approximations to the following types of curves :—

(i) Unimodal or bell-shaped curve, (ii) a negatively accelerated curve, (iii) curve with uniform cycles, (iv) a ladder shaped curve negatively accelerated, (v) an uneven or ambiguous curve.

C. (1) In most cases morning shifts show better work and hence greater efficiency. They are on a higher level and are comparatively straight and uniform. In some cases they also give a negatively accelerated curve. The seventh days in the morning shifts are generally characterized by an upward trend.

(2) The curves of the day shifts assume the shape of simple harmonic motion.

(3) The curves of the night shifts are all ambiguous in shape. The progress and decline are marked with high spurts and deep

depressions. The seventh days show a downward trend. In general morning and day curves keep on a higher level than the night curves. The day curves come nearer morning than night curves in height and shape.

D. Analysis shows that there are depressions in the curve. They are the stages of lowered efficiency and there it is that the disturbing factors including fatigue would appear to operate heavily. The depressed period occupies the major part of the week, e.g.

Group.	Shift.	Percentage of total work.
B	Night	66.3
C	Day	48.53
Z	Night	59.17
P	Morning	48.20

E. The groups when compared with other groups in the same shed and in the same shift, show that those giving better performance in mornings suffer more loss in the night shifts, e.g.

Average output	Group P	Group Q	Group R
Mornings	11,398 lb.	11,354·16	11,455·16
Nights	9,822·83 lb.	9,940·83	9,652·8

The peculiarities call for explanation and attempts are being made to explore their causes. The phenomena deserving most attention are: (i) the plateaus, (ii) depressions and (iii) the sudden spurts. They would seem to define the regions of progress, lowered efficiency or fatigue. A more precise determination of the several factors and the extent of their influences will help in investigating the fatigue phenomena. This done, the methods of counteracting fatigue can be adopted for maintaining a higher level of performance and making the curves more regular.

It may be added ate th end that this is a preliminary report and investigation is in progress. As the work is proceeding, we are collecting data among other things of the optimum output in a 'sample period' of 5 or 7 minutes which would serve as the criterion of performance in the case of each worker or group of workers. This would constitute an essential unit of measurement of work without which the efficiency of the worker cannot be judged. We are going to get this 'unit' from spinning, weaving, carding and other sections of the mill. We are also making careful observations of 'minor accidents' such as lapse of movement, wrong adjustments, failure of attention and above all breakages (of warps and threads, etc.) as indicators of fatigue and lowered efficiency.

Along with this we are carrying out experimental tests of sensory acuities and motor performance by means of the appropriate apparatus and dynamometric tests of relevant workers. We are also issuing a questionnaire with a view to securing information which might be essential for studying auxiliary conditions of work and fatigue. As soon as we have finished at least part of the work here in this mill, we propose to collect data from other mills in the province and other parts of the country.

4. Enquiry on the relationship between noise and output under Professor William Adiseshiah at the St. Stephen's College, Delhi.

I. *The problem.*— The problem chosen for this investigation was the effect of noise on output in the performance of weavers. The particular question to be studied was : Does the elimination of noise associated with the work of a weaver result in any appreciable increase in efficiency ? This problem was investigated in the U.K. by Weston and Adams (Ref. I.H.R.B. Report No. 65) and in their opinion there is an appreciable increase.

II. *General progress of the enquiry.*— The enquiry consists of two main parts :—

(i) A research experiment on weavers in the Mill, involving observations of hourly output and time loss.

(ii) Statistical treatment of data.

Sir Shankar Lall, Managing Director of Delhi Cloth Mills, very kindly permitted one member of the staff and three students of St. Stephen's College, Delhi, to carry out the enquiry in one of the weaving sheds of the Delhi Cloth Mills. A furnished room was provided for this purpose, and the authorities of the mills did much to facilitate the work of the investigators. The enquiry was scheduled to begin on the 1st May, 1947, but it was not possible to get started with the enquiry on that date, as certain essential items of apparatus ordered from the U.K. failed to arrive by that date. Nevertheless, the investigators started on a preliminary study on the performance of weavers, in the hope that as soon as the apparatus arrived from the U.K. the enquiry would begin. This preliminary study began on the 12th May and was carried on till the 25th July. The apparatus did not reach Delhi till the end of July, by which time the investigators had collected data relating to the efficiency and personal factors of a select group of weavers over a period of ten weeks. Weekly statements of the material collected were prepared and sent week by week by by Air Mail to Dr. Thomas Bedford and Dr. S. Wyatt in the U.K., for comment and criticism. Their advice and direction have influenced this work at every step.

By the end of June 1947, it seemed possible to start the main enquiry. The appointment of a research assistant was considered. A young graduate from Allahabad University, Mr. S. K. Ahmad was appointed. He worked from the 1st to the 31st July and resigned on the ground that he found that he was not suitable for the work. A scheme to carry out the experiment connected with the enquiry by enlisting the help of honorary student workers was prepared and was due to come into operation on the 1st September, 1947, but the outbreak of serious disturbances in Delhi made this impossible. At the present moment, the atmosphere in Delhi is *not* conducive to the carrying out of a research experiment of this type.

III. *Work done since the commencement of the enquiry.*— The work done up till now is of a general character, having no direct bearing on the special problem of the enquiry. Nevertheless, the material collected so far is such as to provide a useful background on which the reliability of the data and conclusions of the experiment connected with the enquiry can be tested.

The material now in hand consists of :—

(a) Records of hourly variations in the efficiency of a select sample of weavers over a period of ten weeks.

(b) Records of loss of efficiency of the same group for the same period.

(c) Records of the achievement of the same group in mental tests.

(d) Hourly records of temperature and relative humidity.

(e) Records of interviews with weavers.

The final analysis of the data so far collected is being carried out and a paper entitled "An analysis of variations in the output of cotton weavers" is in preparation. This will be speeded up when the present emergency in Delhi is over.

IV. *The future of the main enquiry.*— All the apparatus necessary for the conduct of the experiment connected with the main enquiry is in hand. The method to be adopted in connection with the experiment as well as the types of statistical computations to be employed have been tried out in the preliminary survey and the student and staff investigators have gained sufficient experience along these lines. As at present constituted, the investigators can undertake this work *in additon to and not in place of* their full routine duties in the college and the university. This makes it necessary for work connected with the enquiry to be carried out during the evening shift in the mills. Until complete calm is restored in Delhi and the curfew is permanently lifted, it will *not* be possible to start on the experiment. The work connected with the enquiry will take at least six months, out of which not less than 3 months will have to be spent inside the weaving shed of the mills. It is doubtful if the enquiry can be continued by the present group of investigators after the 31st March, 1948.

V. *Conclusion.*— The following suggestions are made with regard to the future of the enquiry :—

(1) That the enquiry on the relation between noise and output in Delhi Cloth Mills be given up, as local conditions do not appear to favour the completion of the enquiry before the 31st March, 1948.

(2) That the enquiry be carried out by some other university or college, located in an area where comparatively peaceful and settled conditions of life prevail.

REPORT OF THE FOURTH MEETING OF THE INDUSTRIAL HEALTH ADVISORY COMMITTEE HELD IN NEW DELHI ON THE 10th DECEMBER, 1947.

MEMBERS PRESENT.

Lieut.-Colonel C. K. Lakshmanan, Director, All-India Institute of Hygiene & Public Health, Calcutta. (*Chairman*).

Dr. B. C. Das Gupta, Deputy Director-General of Health Services, Government of India, New Delhi.

Major E. L. Jones, Office of the Director-General of Health Services, New Delhi.

R.B. Dr. M. G. Kini, Superintendent, Stanley Hospital, Madras.

Lieut.-Colonel T. C. Puri, Deputy Director-General of Health Services, New Delhi.

Mr. K. Subrahmanyan, Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. M. N. Rao, Assistant Professor of Industrial and Physiological Hygiene, All-India Institute of Hygiene & Public Health, Calcutta. (*Secretary*).

Mr. S. C. Joshi, Mr. N. S. Mankiker and Major J. Sen were unable to attend.

BY INVITATION.

Major V. M. Albuquerque, Deputy Director-General of Health Services, New Delhi.

Lieut.-Colonel Barkat Narain, Director of Health Services, Delhi.

Mr. A. B. Crawford, Chief Adviser, Factories, Government of India, New Delhi.

Major A. N. Duggal, Director of Public Health, Bihar, Patna.

Mr. T. Gonsalves, Assistant Secretary, Governing Body & Scientific Advisory Board, I.R.F.A., New Delhi.

Dr. C. S. P. Hamilton, Medical Officer, Juri Valley Medical Association and Ross Institute Field Laboratories, Juri, Assam.

Dr. J. M. Orkney, Chief Medical Officer, Women's Medical Service, New Delhi.

Dr. A. R. Mehta, Director of Public Health, Government of East Punjab, Simla.

Mr. D. L. Sharma, Chief Psychologist, Tata Iron and Steel Co., Ltd., Jamshedpur.

Dr. D. K. Viswanathan, Assistant Director of Public Health (Malaria), Bombay Province, Poona.

The Chairman welcomed the members of the Committee and the persons who were present by invitation. He then spoke on the financial position of the Indian Research Fund Association and said that at the present moment there was financial stringency. In view of this he asked the Committee to scrutinize the proposals before them very carefully to ensure that expenditure that could be avoided was cut down.

Item 1.— To consider the work carried out by enquiries sanctioned during the year 1947-48 and to make recommendations regarding the lines of work proposed for 1948-49.

(a) *Industrial Health Research Unit at the All-India Institute of Hygiene & Public Health, Calcutta.*

This unit has just started as it has been difficult to obtain suitable staff. The officers of the enquiry are, as recommended by the Industrial Health Advisory Committee at its meeting held in December 1946, undergoing training in industrial hygiene at the All-India Institute of Hygiene & Public Health, Calcutta. This course started in October and will finish by the end of December. The research work of the unit will, therefore, actually start from the 1st January, 1948. The lines of work proposed are briefly :—

(i) The incidence of lead poisoning in printing presses.

(ii) The relation of environmental warmth to comfort.

(iii) The effects of excess noise on the workers.

The Committee discussed the proposed lines of work and approved them.

(b) *Enquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Professor K. Subrahmanyan.*

The work of this enquiry for the current year has been confined to two jute mills and two printing presses. The results obtained so far are encouraging and Professor Subrahmanyan, therefore, proposes during the next year to extend the enquiry to some more industries. For this an additional technician and a peon are required.

The Committee after discussing the work done on the enquiry recommended its continuance and the additional staff asked for.

(c) *Enquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under Dr. M. A. Rizvi, acting Chief Medical Officer, Batanagar.*

This enquiry has not yet started. The staff of the enquiry has just been selected and it is hoped that the enquiry will start work by the end of December 1947. The Committee approved of the programme of work of the enquiry.

(d) *Enquiry on fatigue and efficiency in paper and textile industries under Professor Kali Prasad at the Laboratories of Experimental Psychology, Lucknow University, Lucknow.*

The work of this enquiry was discussed at length and the general trend of opinion was that the research programme needs modification. The technical staff of the enquiry comprises one research assistant. The work done so far is confined to statistical interpretation of the data collected by the industry itself in one mill. It is proposed during the next year to collect data in a similar manner from the other textile mills in the province and other parts of the country. The Committee felt that Dr. Kali Prasad while conducting the enquiry should maintain contact with the Chairman of the Industrial Health Advisory Committee with a view to modifying the proposed programme of research.

(e) *Investigation on the relationship between noise and output under Professor William Adiseshiah, St. Stephen's College, Delhi.*

The Committee recorded its appreciation of the work done and agreed that the work of this enquiry might be continued by the I.R.F.A. industrial health research unit. The equipment that has been purchased for this enquiry should be made over by Professor Adiseshiah to this unit.

Item 2.—New enquiries.

The Chairman placed before the Committee an application for a grant-in-aid which he had received during the last week from Mr. S. K. Bose, Lecturer in Industrial Psychology, Calcutta University, Calcutta. Mr. Bose regretted that he could not send his application earlier because due to the disturbed conditions prevailing in Calcutta earlier this year it had not been possible to state with any certainty whether the enquiry could be taken in hand. As conditions in Calcutta have now improved he proposes to conduct an investigation into the specific problem of 'traffic accidents and accident-prone personnel' amongst bus and tram drivers in Calcutta. The proposed research is for devising and standardizing suitable tests for accident proneness amongst these workers and these tests would be easily applicable to industrial workers also.

The grant asked for is Rs. 720/- for the part time services of a post-graduate student in industrial psychology.

The Committee was of the opinion that although proposal has been submitted late the enquiry should be recommended for sanction.

Item 3.—To note section (iii) of the minutes of the seventh meeting of the Medical Research Liaison Committee for India held on the 20th March, 1947.

Noted.

Item 4.—The Chairman informed the Committee members about the progress of some of the recommendations made by the Committee during the last Advisory Committee meeting. He specifically referred to the purchase of the Mass Chest Radiographic equipment and stated that relevant information asked for by the Scientific Advisory Board at the last meeting about the cost for running the equipment had been given. He hoped that the funds required would be made available at an early date.

RABIES.

RABIES ADVISORY COMMITTEE.

The Committee met at 10 a.m. on December 9th, 1947.

PRESENT,

Lieut.-Colonel M. L. Ahuja. *(Chairman).*
Dr. K. Banerjee.
Dr. C. B. D'Silva.
Lieut.-Colonel S. D. S. Greval.
Dr. S. R. Pandit.
Dr. R. Sanjiva Rao.
Dr. N. Veeraraghavan.
Dr. K. V. Venkataraman.
Dr. A. G. Brooks. *(Secretary).*

The following were also present :—

Dr. C. G. Pandit.
Dr. B. B. Dikshit.

1. (i) The Committee considered the proposal for an enquiry on rabies by Dr. N. Veeraraghavan at the Pasteur Institute of Southern India, Coonoor.

After a brief discussion Dr. Veeraraghavan withdrew his proposal, as he was in agreement with the generally expressed opinion that his work required independent confirmation, before he proceeded further with it.

(ii) The Committee considered the proposal for an enquiry on the preparation of "quantitatively standardized" antirabic vaccine under Major K. Banerjee at the School of Tropical Medicine, Calcutta. The Committee pointed out that a similar proposal made by Major Banerjee last year was rejected. As no fresh grounds for the proposal had been suggested the Committee were not prepared to recommend the enquiry.

2. The report of Dr. R. Sanjiva Rao on experimental work carried out on "culture vaccine" at Bombay, which had been previously circulated to members, was considered in detail. Dr. Sanjiva Rao showed that Semple's 5 per cent carbolized vaccine had considerable immunizing value, when tested by Habel's technique on mice, and that the culture vaccine was practically valueless. The poor results obtained with the culture vaccine, were attributed by Dr. Veeraraghavan to the deleterious effects of formalin which had been used as a viricidal agent in the vaccines sent to Dr. Sanjiva Rao for test.

Preliminary tests carried out at the Central Research Institute, Kasauli, indicated that culture vaccine was of some value.

Dr. S. R. Pandit informed members that he had tried to culture the rabies virus in accordance with technique described by Dr. Veeraraghavan. His attempts to culture the virus were entirely unsuccessful. It was recommended that all interested workers who had the facilities should carry out similar experiments in culturing the virus and in testing such cultures for their immunizing value.

3. Section 2(ix) of the minutes of the seventh meeting of the Medical Research Liaison Committee for India held at India Office, London, on the 20th March, 1947, was read and noted.

4. The Director of the Central Research Institute, Kasauli, informed members of the Committee that the Pasteur Association of India had generously offered a grant of Rs. 1,50,000 for the purpose of erecting laboratories for anti-rabic research. This offer was subject to a satisfactory arrangement being concluded, either with the I.R.F.A. or the Government of India for the subsequent staffing and maintenance of the centre.

It was resolved that the Government of India be urged to accept this responsibility at an early date to enable the Director of the Central Research Institute, Kasauli, to proceed with the buildings and work.

5. Major Banerjee produced statistical evidence to show that results with one per cent anti-rabic vaccine, as still used at Calcutta, were poor when compared with those of all other Institutes in India where 5 per cent vaccines were used. This was in keeping with animal experiments which similarly proved the value of higher dosage. In view of the provisions laid down in the Drugs Act (1940) it was decided that the Bengal Government be requested to adopt a 5 per cent vaccine in accordance with the standard laid down.

PHARMACOLOGY.

1. Indigenous drugs enquiry under Bt.-Col. Sir Ram Nath Chopra, at the Drugs Research Laboratory, Jammu, Kashmir.

I. Essential Oil Bearing Plants.

The following essential oil bearing plants which grow wild in Kashmir and are reputed for their aromatic properties were investigated during the period under review :—

1. *Elscholtzia Cristata* Wild.—It is a small to medium sized herb with aromatic properties. It grows wild in Himalayas from 1,000 ft. to 9,000 ft. above sea-level along the fields and water channels. In Kashmir it occurs commonly in Sind valley.

The whole plant was collected for investigation and on steam distillation it yielded 0·993 per cent oil with sp. gravity 1·023 at 15° C. and refractive index 1·529 at 20° C.

2. *Heracleum Candicans* Wall.—It is a medium sized herb with long and velvety leaves distributed in the Himalayas from 6,000 ft. to 12,000 ft. above the sea-level. It occurs wild in Pir Panjal and Gurez valley. The roots of the plant were collected from the Pir Panjal side for distillation of essential oil. 1·2 per cent oil was obtained with sp. gravity 1·149 at 15° C. and refractive index 1·453 at 20° C.

3. *Xanthoxylon alatum* Roxb.—It is a shrub or a small tree which grows from 2,000 ft. to 4,000 ft. above the sea-level and is common in Kashmir on J. V. Road below Uri. The bark and fruit are much used in the indigenous system of medicine in fevers, dyspepsia and cholera. It is also used as aromatic tonic. Tooth brushes are made of the branches. The seeds are also used to intoxicate fish. The dry fruit of the plant procured from Muzaffarabad side was steam distilled and 2·01 per cent pale yellow oil with a peculiar odour was obtained having the sp. gravity 1·025 at 15° C. and refractive index 1·475 at 20° C.

4. *Aegle marmelos* Corr.—The tree is indigenous to India and is found wild all over the sub-Himalayan forests in Bengal, South India and Burma. It is very common in the lower forests of Jammu Province. The unripe fruit is regarded as astringent, digestive, stomachic and is said to be an excellent remedy for diarrhœa and dysentery.

Both the dry fruit and leaves were steam distilled. The yield of the oil from the fruits was rather poor but the leaves yielded quite a good quantity of the oil the yield being 0·52 per cent. The sp. gravity was 0·807 at 15° C. and refractive index 1·448 at 20° C.

5. *Prangos Pabularia.*—It is a large perennial herb which grows from 6,000 ft. to 11,000 ft. in the Kashmir valley. The plant is considered to be carminative, diuretic and emmenagogue. The leaves flowering tops and dry roots of the plant procured from Pir Panjal side on steam distillation yielded 0·65 per cent essential oil with sp. gravity 1·129 at 15° C. and refractive index 1·454 at 20° C.

6. *Juniper berries.*—Juniper berries and the oil extracted from them are very ancient remedies and were used for digestive and diuretic purposes. Juniper oil in the commerce is much used for the preparation of the well-known

beverage 'Gin' which owes its characteristic flavour to juniper. There are three species of juniper which commonly grow in Kashmir, namely *Juniper communis, J. macropoda* and *J. recurva.*

7. *Juniperus Communis* Linn. (Vern. Vither).—It is a small dense erect herb which grows at high elevations from 9,000 ft. to 14,000 ft. in the Himalayas more usually on rocky grounds towards the limit of plant growth. The ripe berries of the plant obtained from the Kashmir Sind valley were steam distilled and 0·77% oil with sp. gravity 0·9388 at 15° C. and refractive index 1·448 at 20° C. was obtained.

8. *Juniper Macropoda* Boiss (Vern. Padam).—It is a medium sized tree which grows in Himalayas from 8,500 ft. to 14,000 ft. It is very common in Gurez valley at high elevations. The wood of the tree is much used for pencil making. The dry berries of this plant procured from Gurez valley yielded 3·3 per cent sweet oil with sp. gravity 0·8571 at 15° C. and refractive index 1·473 at 20° C.

II. Pharmacopeial Plants and Substitutes.

1. *Hyoscyamus niger leaves.*—It was reported that the alkaloidal percentage of the *Hyoscyamus niger* leaves growing in Kashmir and other places in India is not upto the B. P. standard. In order to ascertain the above facts collection of series of both the cultivated and wild variety of the *Hyoscyamus* leaves was done from different places in Kashmir and from different places in India i.e. Lyallpur, Saharanpur and N.-W.F.P. It has been observed that the alkaloidal percentage of the *Hyoscyamus* leaves of the both cultivated and wild variety from the Kashmir vary from 0·066 to 0·084 per cent and those obtained from other places was below the B.P. standard.

2. *Effect of storage on pyrethrum.*—In order to study the effect of storage on pyrethrum flowers samples from the same commercial lot were kept at the following places in the state :—

(i) *Baramulla.* Height 5,000 ft. above sea-level and annual variation of temperature 28° F. to 100° F.

(ii) *Srinagar.* Height 5,000 ft. above sea-level and temperature variations—29° F. to 100° F.

(iii) *Jammu Tawi.* Height 1,000 ft. above sea-level and annual temperature variations from 40° F. to 119° F.

(iv) The 4th sample was kept in the cold storage of the laboratory temperature variation generally between 32° F. to 40° F.

Samples of 1945 pyrethrum crop were analysed in the 3rd week of October, 1945, i.e. in the beginning of the winter season. These samples were again analysed in June 1946 having remained under above conditions of storage for the winter season. It was observed that practically very little deterioration had taken place in all the samples in winter.

Analysis of this was again done in February 1947 and it was seen that in the case of pyrethrum samples kept at Jammu the deterioration was maximum, less so in the case of samples kept at Srinagar and Baramulla and the least in the cold room.

This experiment is in progress and further observations will be communicated in latter reports.

3. *Rhamnus Virgatus* :—The investigation was started to find out the substitute for the official cascara sagrada bark (*Rhamnus purshiana*). Chemically it was found to be inferior to the standards laid by the B.P. The experiments were conducted during the present year on puppies. It was found that the species was not suitable as a purgative. The animals did not exhibit any effect on the bowels even with as much as double the dose of the official plant. The work will, however, be continued with soft extract of the plants on guinea-pigs etc.

III. Indigenous Drugs.

1. *Senecio Jacquemontianus Bentham* (Vern. Hatermool).—It is a long herb with roots having aromatic odour. The plant is common in Himalayas from 10,000 ft. to 13,000ft. and in Kashmir it is common in Gurez valley. In the indigenous system of medicine the plant is used in skin diseases. The plant is also known to be poisonous and produces hepatic cirrhosis in live-stock. The plant has not been investigated before. For a systematic study 50 g. of powdered dry roots were successively extracted with various solvents. The following percentages of the extra acts were obtained after removal of the solvent and drying the residue at 100°C.

	Per cent.
Petroleum ether ..	10·9
Ether sulphuric ..	0·94
Chloroform ..	1·04
Ethyl acetate ..	0·64
Alcohol ..	12·5

The roots gave 1·2 per cent essential oil on steam distillation. The various extracts were carefully examined with a view to ascertain whether or not any substance of glucosidic, phenolic, resinous or alkaloidal character and likely to be physiologically active were present. The petroleum ether extract and the steam distillation of the roots gave sweet smelling essential oil. The chloroform extract showed the presence of alkaloidal which gave flocculent precipitates with alkaloidal reagents, i.e. Mayer's reagent, Karaut's reagent, picric acid solution, iodine solution, Schibler's reagent, etc. The yield of the crude alkaloidal residue from the roots amounted to about 0·1 per cent. Further work on the isolation and purification of the alkaloids is in progress and shall be communciated in the next report.

2. *Scoparia Dulcis* Linn.—It is a herb which grows wild in Assam and East Bengal. It is used in ague, toothache and excessive menstruation. The drug is also considered astringent, emetic, mucilaginous and emollient. For investigation purposes the herb was procured from Chittagong. The whole dried plant was successively extracted with various solvents and the following percentages were obtained :—

	Per cent.
Petroleum ether	1·5
Ether	0·82
Chloroform	0·63
Ethyl acetate	0·63
Ethyl alcohol ..	3·5

The various extractives were examined with the following results :—

Petroleum ether extractives consisted mainly of chlorophyll wax and oil. Ether extract was greenish in colour and contained chlorophyll and resins. Chloroform extract showed the presence of a substance of alkaloidal nature. Ethyl acetate extract did not contain any thing except resinous type of substances. Alcoholic extract contained large amount of tannins and colouring matter.

For the isolation of the basic constituent of the drug the whole dried plant was percolated with alcohol. The syrupy residue contained after concentrating the alcoholic extract in vacuo was stirred, in 1·0 per cent sulphuric acid. The aqueous solution thus obtained was freed from non-basic impurities by extraction with chloroform. This was made alkaline with ammonia and extracted with chloroform. The chloroform solution of the base was dried over sodium sulphate and the solution recovered. A very small yield of the basic substance was obtained which was tested with various alkaloidal reagents. The base gave no precipitate with Mayer's reagent, Schibler's reagent, picric acid and iodine solution precipitate was obtained with potassium bismuth iodide. The yield of the base was so small that no further examination could be undertaken.

The above results indicate that the medicinal properties of the drug are due to the presence of a substance of alkaloidal nature but the quantity present is so small that the drug cannot possess any marked degree of therapeutic activity.

OTHER RESEARCHES.

1. Filterable viruses enquiry under the Director, King Institute, Guindy, Madras.

I. Relative Efficiency of Several Methods of Concentrating Influenza Virus.

Various methods have been devised to concentrate influenza virus so as to secure a more potent preparation for use as an immunising vaccine. Thus Chambers and Henle (1941) showed that influenza virus in the chorio allantoic fluid can be concentrated and partially purified by precipitation with protamine. Hirst, Rickard and Whitman (1942) and Hare ; McClelland and Morgan (1942) demonstrated that the precipitate which forms when the infected chorio allantoic fluid is frozen and then thawed contains the bulk of the active virus and this method can be used for purposes of concentration. Bodily, Corcy and Eaton (1943) used alum while Salk (1945) and Stanley (1945) used calcium phosphate for precipitation and concentration. In addition, Hirst (1941) and McClelland and Hare (1941) have shown that influenza virus in infected chorie-allantoic fluid can be absorbed by the red cells of the embryo or by adult chicken erythrocytes, and that under proper conditions much of the virus could be eluted from the red cells. These observations formed the basis of the method of concentration and purification of influenza virus by Francis and Salk (1942). Cox, Scheer, Aiston and Bhonel (1947) have concentrated and purified influenza virus from the infected chorio-allantoic fluid by precipitation with methyl alcohol at low temperatures.

However, the method most commonly employed in the preparation of the vaccine for use in the field was that of Francis and Salk (1942) utilising the phenomenon of absorption and elution of the virus from fowl red cells. The other methods described above, no doubt yield concentration of the virus suitable for the manufacture of vaccine for field use but no observations have so far been made on the antigenicity of the fresh concentrated vaccines. It was decided to undertake studies in all these respects.

The following methods of concentration are being studied :—

(a) Precipitation with alum.

(b) Precipitation with calcium phosphate and the subsequent purification.

(c) Concentration and purification with methyl alcohol at 4° C., and

(d) Concentration by absorption on fowl red cells and subsequent elution into normal saline.

Apart from the concentration of the virus units in any given volume, the purification effected by the removal of non-specific protein as determined by the nitrogen content of the vaccine was also studied.

Results.—Table I shows the results of the R.C.A. activity and nitrogen content of different vaccines prepared :—

TABLE I.

Infected allantoic fluid untreated.	Concentrated virus vaccines infected allantoic fluid untreated.							
	Red cell eluate.		Alum pptn.		Alcohol pptn.		Cal. phcs. pptn.	
Aggln. titre.	N-content mg./ml.	Aggln. titre.	N-Content mg./ml.	Aggln. titre.	N-C. mg./ml.	Aggln. titre.	N-C mg./ml.	Aggln. titre.	N-C. mg./ml.
1 : 256 — 1 : 512	0·75	1 : 2048 — 1 : 4096	0·42	1 : 2048 — 1 : 2006	0·47	1 : 2048 — 1 : 4096	0·24	1 : 2048 — 1 : 4096	0·3

Calcium phosphate precipitate gives the virus in the purest form as judged by its N-content of the alcohol precipitation being 0·24 mg./ml. Red cell eluate being 0·42 mg./ml., and the alum precipitation 0·47 mg./ml. The tests for antigenicity are under way.

If the calcium phosphate precipitate is antigenically superior of equal to other vaccines it must be the method of choice for the manufacture of the vaccine as higher dosage of the vaccine can be given with lesser reactions.

II. REACTION BETWEEN INFLUENZA VIRUS AND THE SUSCEPTIBLE TISSUE CELLS.

Joyce D. Stone (*Nature*, **159**, 7th June, 1947) has carried out investigations in order to determine whether the receptor-destroying enzyme of *V. choleræ* could alter the reactivity of tissue cells normally susceptible to infection with influenza virus. It has been found that the enzyme interferes with the absorption of the virus hæmagglutinin on the susceptible cell in the allantoic cavity of the chick embryo. In the control which did not receive a pre-treatment with the enzyme, it was found that there was a rapid absorption of virus followed by liberation after a period of three to four hours.

The observations recorded above were confirmed. The results obtained were as follows:—

The eggs which received a pre-treatment with *V. choleræ* enzyme, the virus, was not absorbed, the receptor-destroying enzyme preventing this absorption. But in the case of the control eggs, which did not receive a pre-treatment with the enzyme, there is a rapid absorption of virus followed by liberation after a period of three to four hours.

Since the absorption recorded above are of fundamental importance, it was decided to study whether the same phenomenon could be observed by the tissue-culture technique employing for the purpose flask-culture methods of culting minced chick-embryo tissues. Details of the technique are not described here. The results obtained were as follows (Table II):—

TABLE II.

Tissue Culture Flask No.	Titration of the samples at the end of				
	30 mts.	60 mts.	120 mtes.	180 mts.	240 mts.
I	1 : 64	1 : 96	1 : 96	1 : 96	1 : 96
I (Control)	1 : 4	1 : 16	1 : 64	1 : 96	1 : 96
	(The titre of the inoculum used was 1 : 128/1 : 256)				
II	1 : 96	1 : 192	1 : 128	1 : 192	1 : 192
II (Control)	1 : 8	1 : 32	1 : 64	1 : 128	1 : 128
	(The titre of the inoculum used was 1 : 256/1 : 512)				

It would appear that even in the flask culture technique it is posible to obtain the prevention of the adsoprtion of influenza virus after treating the cells to the action of an enzyme obtained from the *V. choleræ*. The relative merits of the two methods are now being studied in order to investigate several allied problems in this respect.

III. PREPARATION OF INFLUENZA ANTIGENS FOR DIAGNOSITIC TESTS.

Virus A and virus B influenza antigens have been prepared. Since sera from cases of influenza were not available for study, it was considered advisable to examine the sera from suspected cases of enteric received in this Institute for routine examinations in order to determine their agglutination titre against both A and B viruses. The agglutination could yield data to determine the diagnostic titres significant of influenzal infections when such sera are received for test. The results were as follows (Table III):—

TABLE III.

Agglutination inhibition titre of human sera with influenza viruses A & B.

Particulars.	Below 64	Titre.					Total.
		64	128	256	512	1,024	
Influenza IR 8 A Strain	3	8	15	9	6	3	44
Influenza B Lee Strain	7	5	8	8	3	2	33

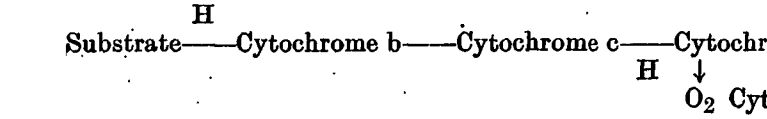
The observations will be continued on sera from other cases of fever and on normal sera.

IV. The strain of vaccine lymph cultivated on the chorio-allantoic membranes of developing chick-embryos is being maintained.

2. Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta.

1. *Investigations on the inhibitor of the cytochrome oxidase system, (Warburg's respiratory enzyme) in cobra venom.*

In the previous report, evidence was recorded regarding the presence of a separate active principle in cobra venom, responsible for a marked inhibition of the cytochrome-cytochrome oxidase system. The transfer of hydrogen from the substrate molecule to oxygen by the cytochrome oxidase system proceeds as follows :—



The inhibition of this particular oxidase system may be due to the action of the venom on any of the three cytochromes or on cytochrome oxidase or both. To clarify this point, a sample of cytochrome c was isolated from ox heart in a concentrated form by the method of Koilin and Hartree (*Proc. Roy. Soc. B.*, 1937, 122, p. 298), and several experiments were arranged in the following way. In each of four Warburg's flasks A, B, C and D containing phosphate-Ringer-Locke solution of pH 7·4, were weighed out approximately 50 mg. portions of freshly chopped and minced pigeon's brain. To the brain suspension in the flasks B and D, were added 0·1 mg. of venom dissolved in phosphate-Ringer. 0·5 c.c. of 1 per cent p-phenylene diamine hydrochloride solution in phosphate-Ringer brought to pH 7·0 was kept in the side bulb of all the flasks, whilst 0·1 c.c. of a concentrated solution of cytochrome c was kept in the side bulbs of the flasks C and D in addition to the substrate. 0 2 c.c. of a 20 per cent KOH solution was kept in the inner cup of each of the flasks together with a roll of filter-paper. The flasks were fitted to the manometers and shaken for 1 hour and 30 minutes at 37·5° C. ± 0·1° C. in a thermostat bath in an atmosphere of air. The solutions from the side tubes were then tipped off into the main compartment of the flasks, the taps were then closed and the oxygen consumption was determined at intervals of 5 minutes for a period of one hour. Several experiments with different amounts of the cytochrome c solution were carried out. Thus, in a typical experiment, it was found that in the absence of added cytochrome c, the oxygen consumption per gramme of brain (wet basis) in 30 minutes with and without the venom were respectively 240 cu.mm. and 1,620 cu.mm. whereas the figures for oxygen consumption per gramme of brain (wet basis) in 30 minutes in the presence of added cytochrome c, with and without venom were respectively 280 cu.mm. and 1,820 cu.mm. The experiment was repeated and similar results were obtained in each case. These results clearly indicate that addition of cytochrome c does not appreciably influence percentage inhibition of the oxygen uptake of brain with p-phenylene diamine after the venom has acted upon it. The inhibition of the cytochrome oxidase system is, therefore, due to the action of the venom on cytochrome oxidase, which includes also the carriers cytochrome b and a.

In this connection, it should be noted that the well-known inhibitors of cytochrome oxidase, e.g. HCN, CO, H_2S and axide produce their effects by forming complexes with the iron of the respiratory enzyme. It remains to be

seen whether the inhibition by cobra venom is produced in a similar manner or differently.

Further investigations are necessary to elucidate this point.

Isolation of the inhibitor :

Attempts were made to isolate the inhibitor referred to above from cobra venom. After many experiments, the following procedure was adopted :—

To 100 c.c. of a solution of cobra venom in distilled water adjusted to pH 9·0, were added with stirring 20 grammes of NaCl in small amounts at a time. The precipitate that was formed was filtered off. It was found that the major portion of the activity remained in the filtrate. The precipitate, after washing with 20 per cent NaCl solution, was rejected. The filtrate together with the washings was brought to pH 2·0 with sulphuric acid solution, when a second precipitation occurred. Most of the activity was found in this precipitate. After allowing to settle for 30 minutes, it was filtered off and dissolved in 50 c.c. of water at pH 3·8 and 8 grammes of anhydrous Na_2SO_4 were added slowly with continuous stirring. The precipitate formed was filtered off and found to be inactive. The precipitate was washed with 16 per cent Na_2SO_4 solution and rejected. The filtrate and the washings were combined and the volume measured. Sufficient Na_2SO_4 was then added to bring the concentration of the salt to 22 per cent. A precipitate was formed and it was found to be active. It was filtered off, dissolved in 50 c.c. of water at pH 3·8 and 11 grammes of anhydrous Na_2SO_4 were again added slowly. This precipitate was filtered off and dried. Its protein content was determined from nitrogen estimation. Weight for weight this protein was found to be about 10 times more active in inhibiting respiration than the dry cobra venom. The toxicity of the protein, as tested by intramuscular injection into pigeons, was found to be about 0·14 mg.

Further investigations are being carried out to obtain the inhibitor in a purer state.

2. *On the inhibitor of glycolysis in cobra venom.*

It has been previously reported that cobra venom produces a strong inhibition of glycolysis occurring in tissues. Attempts were made to isolate the inhibitor. A method has been developed for partially purifying this inhibitor and is described below :—

To 100 c.c. of a 1 per cent solution of cobra venom dissolved in distilled water and adjusted at pH 6·0, were added 30 grammes of pure NaCl slowly. A precipitate was formed which was found to be inactive. It was filtered off and washed. The filtrate and the washings are combined and brought to pH 2·0 when an active precipitate appeared. This was allowed to settle at 38°C. for 30 minutes and filtered off. The precipitate was dissolved in 50 ml. of water, the pH adjusted at 4·0 and 8 grammes of anhydrous Na_2SO_4 were added in small amounts at a time. The precipitate was found to be active. After filtration the precipitate was dried and its activity tested. The activity was found to be about 60 per cent and its protein content 11 per cent with respect to crude cobra venom.

Further work is being carried out in this line.

3. *Effect of banded krait venom on tissue respiration with glucose.*

In continuation of the work on tissue respiration, the effect of banded krait venom on the oxidation of glucose by pigeon's brain tissue was investigated.

About 100 mg. portions of freshly chopped and minced pigeon's brain tissue were weighed out in Warburg's flasks and suspended and broken up in phosphate-Ringer-Locke solution pH 7·4. Adequate amount of the krait venom dissolved in phosphate-Ringer was added to the brain suspension. Suitable controls were also kept. 0·2 c.c. of a 20 per cent KOH solution was kept in the inner cup together with a roll of filter-paper. The flasks were fitted to the manometers and incubated at 37·5°C.±0·1°C. for 1 hour and 30 minutes in an atmosphere of air. By this process, the venom was allowed sufficient time to exert its effect on the brain and also the tissue was depleted of a portion of the substrates present in it. Next 1·0 c.c. of a 0·5 per cent solution of glucose in Ringer was added to the brain suspension and the respiration was followed for 1 hour at intervals of 15 minutes.

Various doses of krait venom from 0·5 γg. onwards have been used. It has been found that 0·5 γg. of the venom has no effect on the oxidation of glucose, whereas 1·0 γg., 2·0 γg. and 4·0 γg. produce an inhibition of 40 per cent, 80 per cent and above 80 per cent, respectively.

Further investigations on the effect of venoms of bended krait, common krait, *Vipera russellii* on the oxidation of biologically important metabolites like lactic acid, pyruvic acid, succinic acid, ascorbic acid (for cytochrome oxidase) are in progress.

3. Enquiry into methods for the manufacture of penicillin and other problems connected with it under the Director, Haffkine Institute, Bombay.

1. *Study of the nature of the penicillin or penicillins produced by different strains of* Penicillium chrysogenum *under different conditions.*—It is now known that different strains of *P. chrysogenum* produce different penicillins depending upon the conditions of growth of the mould and also the composition of the medium employed. To obtain accurate date on this issue, three of the best strains of *P. chrysogenum* evolved in U.S.A. called Q176, X-1612 and NRRL 1984, N22 were taken up for study in the first instance. *P. chrysogenum*, Q176 and X-1612, are strains developed by mutation with X-ray and ultraviolet rays and they give the highest yields of penicillin, the former being now used in industry for the manufacture of penicillin. The strain NRRL 1984, N22 is an ultra-violet mutant developed in Peoria and claimed to give about 50% of penicillin X. These strains were brought from U.S.A. and preserved in the lyophilized form. Tubes are taken, regenerated and cultures are made on sporulating agar in Roux bottles. These spores are used for the inoculation of the media. Each culture is not subjected to more than four or five subcultures because they tend to mutate into strains with low penicillin producing power. The media used is of the following composition:

Lactose monohydrate	27·5 g.
Sodium nitrate	3·0 g.
Magnesuim sulphate, $7H_2O$	0·25 g.
Potassium dihydrogen phosphate	0·50 g.
Zinc sulphate	0·44 g.
Manganese sulphate	0·02 g.
Glucose	3·0 g.
Concentrate corn steep liquor (Staley's)	40 c.c.
Tap-water	1 litre

In one set of batches no phenylacetic acid was added and in the other 0·3% of phenyl acetic acid was added.

The media were distributed in Roux bottles (175 c.c. per bottle), inoculated with the spores and incubated in the flat position at 23°C. to 24°C. In each batch 40 to 90 bottles were used.

The maximum potency of the culture fluid was reached on about the 8th day. The culture fluid was collected and the penicillin extracted as follows: The culture fluid was cooled to about 5°C., mixed with about one-third the volume of butyl acetate and the solution acidified with 10% phosphoric acid to pH 2·0 to 2·5. After thorough mixing, the mixture was centrifuged and the butyl acetate separated. This was washed twice with 1/10 the volume of distilled water. The butyl acetate was then extracted with 5% sodium bicarbonate at pH 7·4. The bicarbonate extract was then cooled, acidified to pH 2·0 to 2·5 with phosphoric acid and extracted with one-third the volume of butyl acetate. From this solvent penicillin is taken up into 1·5% sodium bicarbonate solution at pH 7·4. This final solution is stored in refrigerator at −25°C. or dried by the lyophilic method in a Strumia machine. The above procedure is designed to extract all the different penicillins from the culture medium.

The final solution obtained was then chromotographed over activated alumina (Brockmann) using other as the solvent and for developing the chromatogram. The alumina was eluted with phosphate buffer of pH 7·4 and the eluate was dried. The chromatographic purification was repeated if necessary. The final product obtained is then analysed for the penicillin by the "counter-current distribution" method.

So far seventeen batches were worked up and the purified products are being analysed by the counter-current distribution method to identify the penicillins contained in them. The potencies of the culture fluids varied between 100 to 200 units per c.c. and the final concentrated solutions averaged 2,000 units per c.c.

2. *Rapid titrimetric method of assay of penicillin.*—Since the biological methods of assay (the cup method and the serial dilution method) take about one day to complete and also requires experience in bacteriological techniques for carrying out, rapid chemical assay methods were tried. The iodimetric titration method, outlined by the Squibb Institute, was investigated and has now been standardised by working with crystalline penicillins G, X and K. The method is as follows:—

A weighed sample of penicillin is dissolved in water and an aliquot portion taken so that it contains usually about 1,000 to 5,000 units of penicillin. It is acidified in the cold with phosphate buffer of pH 2 and the penicillin liberated taken up in chloroform. The chloroform extract is made up to a known volume (50 c.c.). Ten c.c. of this chloroform solution are mixed with 10 c.c. of N/100 iodine solution and after a few minutes, the residual iodine is estimated by titration with N/100 sodium thiosulphate solution. Another 10 c.c. of the chloroform solution are shaken with 10 c.c. of N sodium hydroxide for about 15 minutes, the alkali then neutralized by adding 10·2 c.c. of N hydrochloric acid and the residual iodine estimated by titration with N/100 sodium thiosulphate solution. The difference between the two titration gives the quantity of iodine consumed by the penicillin inactivated by alkali. Since each molecule of penicillin takes 8·9 atoms of iodine, the quantity of penicillin in the sample is calculated. The results are checked by taking a known weighed amount of

crystalline penicillin and estimating the penicillin content by this method. This method has been found to be very reliable and the estimation takes about one hour at the most. The minimum amount that could be reliably estimated is about 500 units; below this the errors in titration become predominant. With this method, commercial samples of penicillin, penicillin pastes, and the concentrated solutions of penicillin in the process of extraction have been estimated, the results checking up the results obtained by the biological methods. This method is particularly valuable in estimating the penicillin content of samples containing antiseptics besides penicillin.

3. *Differential assay of penicillin G and K.*—Since the strains of *P. chrysogenum* (Q176) now being used for the manufacture of penicillin produce penicillin K also in addition to penicillin G, the assay of the percentage of penicillin K which therapeutically is not active, in any commercial sample of penicillin is of importance. A combined titrimetric and chromatographic method (using alumina) was tried and found to yield erratic results. Next differential separation of the two penicillins at different pH from the solvent was tried. Though it is believed that penicillin G is extracted from the solvent by buffer of pH 6·4 while penicillin K was not extracted by this but by buffers of 7·4 only, a careful study of this using crystalline penicillins G and K and the solvents chloroform and butyl acetate, disclosed that the differential separation method does not give accurate results. This is being further studied using various other solvents.

4. *Counter-current extraction method to estimate different penicillins.*—Using the "counter-current extraction" apparatus obtained from U.S.A. and adopting the technique of Dr. Craig, samples of crystalline penicillins G, X and K were analysed to obtain standard curves for these. First, attempts were made to see if 10-mg. samples could be used for the analysis; but the errors in weighing were too great. Very satisfactory results are obtained when samples varying from 50 mg to 100 mg. are used. Attempts are being made to try the iodine titrimetric estimation in the place of weighing the residues. This method enables one to accurately assay the percentages of the various penicillins in a given sample. With this, the crystalline penicillin G, X and K and also the commercial crystalline penicillins have been analysed.

V. INDIAN JOURNAL OF MEDICAL RESEARCH and INDIAN MEDICAL RESEARCH MEMOIRS.

1. (a) *The Indian Journal of Medical Research, Volume XXXIV*, 1946.—Only two numbers of the *Journal* were published and these appeared in May and October, respectively. The new style of printing with narrower margins was continued in the interests of economy. The average size of each number was 163 pages of printed and tabular matter, with two plates, representing an increase of 3 pages per number as compared with Vol. XXXIII.

(b) *The Memoirs.*—No *Memoir* was published with Volume XXXIV as no material was received.

(c) *Cost.*—The net cost of publication of Volume XXXIV (two numbers only) was Rs. 4,456.

2. (a) *The Indian Journal of Medical Research, Volume XXXV*, 1947.—With this volume publication of quarterly issues has been resumed. Three numbers, i.e. the January, April and July 1947, have already appeared. Slight delay occurred in the appearance of the January number, while the July number was delayed by 2 months. This was due to civil disturbances all over the country. The April number, however, appeared in time. January issue contained 39 pages, April issue 75 pages and July issue 120 pages with 8 plates. October issue is still in Press. Volume XXXV will be somewhat smaller than Volume XXXIV. The cause of the smaller size of this volume is due to paucity of material received during the last few months.

(b) *The Memoirs.*—As no suitable material was received, no *Memoir* was published with this volume.

3. *Distribution* (1946) (Figures for 1947 will be given next year).

Indian subscriber ..	87		
Foreign subscribers ..	132	=	219
Exchange list	188		
Free list	131	=	319
Voucher copies to advertisers, regn., make-up, police, specimen, propaganda, etc... ..		=	35
Balance left with publishers for sale		=	127
Total printed ..			700

4. *Distribution Lists.*—The existing lists were last revised in July 1938. The revision of these lists is in hand but will not be completed before end of the year.

5. *Number of copies printed.*—Only 700 copies of the *Journal* have been printed since 1940 instead of the previous 900. This number is considered sufficient to meet all current demands. Volumes I-VIII of the *Journal* are out of print.

6. *Cost of printing the Journal.*—The following statement shows the cost of production during the last five years:—

Cost of printing.

Number.	XXX 1942	XXXI 1943	XXXII 1944	XXXIII 1945	XXXIV 1946
April ..	Rs. 2,949	May 2,633	May 2,116	May 4,037	May 4,086
July ..	Rs. 1,650				
October ..	Rs. 2,413	2,486	2,752	2,777	2,778
January (next) ..					1,447
Totals ..	Rs. 7,012	5,199	4,868	6,814	8,311
Average pages ..	158	125	126	160	163

Recoveries.

April ..	Rs. 1,034	May 837	May 1,293	May 1,842	May 1,204
July ..	Rs. 946				
October ..	Rs. 1,518	880		1,580	1,204
January (next)					*Nil.*
Totals ..	Rs. 3,498	1,717	1,293	3,422	2,408

7. *Net-cost of Journal and Memoirs* :—The following statement shows the net cost of the production of the *Journal* and *Memoirs* during the last five years :—

Journal.

Number	1942	1943	1944	1945	1946
April	Rs. 1,915	May 1,796	May 823	May 2,194	May 2,882
July ..	Rs. 704				
October ..	Rs. 895	1,606	2,752	1,197	1,574
January (next)					1,447
Totals ..	Rs. 3,514	3,402	3,575	3,391	5,903

For convenience of accounting, all receipts are credited to the *Journal* account, and the figures given for the *Memoirs* are the actual cost of production.

8. *Financial position for the year* 1947-48.—The budget for the year provided for Rs. 14,000 to meet the cost of four quarterly numbers of the *Journal* and Rs. 2,000 for any *Memoir* which may be published. The following are the actual and estimated expenditures :—

I. *Actual :*

Volume XXXV, No. 2 (April 1947) .. .. Rs. 1,569

II. *Estimated :*

Volume XXXV, Nos. 3 and 4 (July and October 47)
Volume XXXVI, No. 1 (January 1948) @ 3,000 each .. .. .. } = Rs. 9,000

Total .. 10,569

9. *General*:—The editorial policy of the *Journal* was revised in 1945 on the recommendations of the Scientific Advisory Board and the Editorial Board was enlarged to include 25 referees. A note for the guidance of members of the Board was also prepared by the Editor and circulated to all concerned. The services of the members of the Board have been fully utilized in accordance with the policy adopted. The number of members on the Editorial Board has considerably fallen now with many officers having retired and proceeded to the U.K.,

As decided by the Scientific Advisory Board publication of quarterly issues of the *Journal* has been resumed with effect from January 1947. Now that the paper shortage is no longer acute, we have now reverted to the old style of printing with wider margins and narrower columns.

All the papers published in Vol. XXXV were submitted by workers in India or were the results of investigations carried out in India. The lines of special research activity are reflected in the nature of papers received. Of the 25 articles which have been published or accepted for publication 14 were on nutrition, 7 on bacteriology, 2 on biochemistry, 1 on rabies and 1 on snake venom.

The size of the *Journal* has been considerably reduced owing to reduction in the number of papers received for publication.

VI. INDIAN JOURNAL OF MALARIOLOGY.

The Journal of the Malaria Institute of India, Volume VI, Nos. 3 and 4, 1946.—These two numbers of the *Journal* with a total of 260 pages of printed and tabular matter with 4 plates, representing an increase of 8 pages *vis a vis* the two previous numbers, appeared in June and December. The style of printing with narrower margins adopted during the war emergency was continued due to paper shortage.

The net cost of publication of Volume VI (two numbers) was Rs. 2,720.

The Indian Journal of Malariology, Volume I.—The title of the *Journal* was changed to "*Indian Journal of Malariology*" with effect from March 1947, as desired by the Governing Body of the Indian Research Fund Association.

The *Journal* was edited by Major-General Sir Gordon Covell up to the 28th February, 1947. From 1st March to 9th August, 1947 it was edited by Lieut.-Colonel M. K. Afridi, and since the latter date the editorship was taken over by Lieut.-Colonel Jaswant Singh.

Publication of the quarterly issues of the *Journal* was resumed with effect from March 1947. Now that the paper shortage is no longer so acute, the pre-war style of printing with wider margins and narrower columns has been reverted to. The March number consisting of 230 pages and the June number with 115 pages have already appeared, and all papers published were submitted either by workers in India or were the results of investigations carried out in India. Owing to the disturbances, strikes and dislocation of communications there was some delay in the publication of the March and June numbers.

Distribution.—With the termination of the war, demand for the *Journal* has increased, and the number of printed copies per issue has consequently been increased from 400 to 600. The number of copies distributed are as under:—

Indian subscribers	112		
Foreign subscribers	48	=	160
Exchange list	148		
Free list	97	=	245
Voucher copies to advertisers, specimens, propaganda etc.			42
At the Malaria Institute for distribution			20
Balance left with the publishers for sale			133
Total printed			600

VII. Publications on medical research under the auspices of the Indian Research Fund Association during the year 1947.

A. *Indian Journal of Medical Research* and *Indian Medical Research Memoirs.*

1. ADISHESHAN, R., PANDIT, C. G., and VENKATARAMAN, K. V.—Statistical Evaluation of Anti-cholera Inoculation as a Personal Prophylactic against Cholera and its Efficacy in the Prevention and Control of Cholera Epidemics (Vol. 35, No. 3).
2. BRAGANCA, B. M., and RAO, M. V. RADHAKRISHNA.—Hypoprothrombimæmia produced by Sulphathiazole in Rats on a Diet free of Vitamin K and cured by Synthetic Vitamin K (Vol. 35, No. 1).
3. CHANDRA SEKAR, C.—Statistical Assessment of the Efficacy of Anti-cholera Inoculation from the Data of 63 *Cheris* in South Arcot District (Vol. 35, No. 3).
4. DATTA, N. C.—Plasma Proteins in Health and Disease. Part II. Fractionation of Plasma Proteins in Hyperproteinæmia (Vol. 35, No. 4).
5. DATTA, N. C., and CHAKRAVARTY, R. K.—Plasma Proteins in Health and Disease. Part I. Plasma Proteins in Healthy Individuals (Vol. 35, No. 4).
6. DAY, C. D. MARSHALL.—Antiscorbutic Deficiency during Lactation as a Cause of Infantile Scurvy and Dental Dystrophies (Vol. 35, No. 3).
7. DAY, C. D. MARSHALL, and SHOURIE, K. L.—The Effect of Pregnancy on the Incidence of Dental Caries in Indian Women (Vol. 35, No. 2).
8. DAY, C. D. MARSHALL, and SHOURIE, K. L.—Hypertrophic Gingivitis in Indian Children and Adolescents (Vol. 35, No. 4).
9. DIKSHIT, P. K., and PATWARDHAN, V. N.—Studies in Experimental Rickets: The Alkaline Serum Phosphatase in Rachitic Albino Rats (Vol. 35, No. 2).
10. SADANA, J. C., and BASHIR AHMAD.—The Carotenoid Pigments and the Vitamin A Activity of Indian Carrots (Vol. 35, No. 2).
11. SHOURIE, K. L.—A Dental Survey in Ajmer-Merwara (Vol. 35, No. 3).
12. SHRIVASTAVA, D. L., and BRUCE WHITE, P.—Note on the Relationship of the so-called Ogawa and Inaba Types of *V. cholerœ* (Vol. 35, No. 3).

B. *Indian Journal of Malariology.*

1. AFRIDI, M. K., RAMAKRISHNAN, S. P., GOSWAMI, A., and MENON, M. K.—An Enquiry on certain Aspects of Paludrine (Vol. 1, No. 15).
2. AFRIDI, M. K., and BHATIA, M. L.—Malaria Control of Villages around Quetta (Baluchistan) with D.D.T. (Vol. II, No. 19).
3. AFRIDI, M. K.—A Critical Review of the Therapeutic Trials on Paludrine carried out in India (Vol. III, No. 25).
4. AFRIDI, M. K.—A Scheme for the Control of Malaria in Villages in Delhi Province (Vol. IV, No. 36).
5. DAKSHINAMURTY, S.—Measurements of Malaria (Vol. II, No. 22).
6. COVELL, G.—The Malaria Institute of India, 1933-47 (Vol. I No. 1).
7. COVELL, G.—A Brief Review of the History and Development of the more Important Antimalaria Drugs (Vol. II, No. 16).

8. COVELL, G.—Bats, Clover, Water Lettuce and Malaria (Vol. II, No. 17).
9. MULLIGAN, H. W., and SOMMMERVILLE, T.—Malaria in the Malabar Squirrel (Vol. II, No. 23).
10. PURI, I. M., and RAJINDAR PAL.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part I (Vol. I No. 10).
11. PURI I. M., and KRISHNASWAMI, A. K.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part II (Vol. I, No. 11).
12. PURI I. M., and BHATIA, M. L.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part III (Vol. I No. 12).
13. PURI I. M., and KRISHNASWAMI, A. K.—Studies on some Insecticides against Anopheline Adults and Larvæ. Part IV (Vol. I, No. 13).
14. PURI, I. M.—The Practical Application of DDT for Malaria Control on Rural and Urban Areas in India (Vol. I, No. 14).

C. *List of papers published by workers under the I.R.F.A. in Journals other than the Indian Journal of Medical Research and the Indian Journal of Malariology.*

1. CHAUDHURI, R. N., and RAI CHOWDHURI, M. N.—Clinical Trials of Paludrine (*Ind. Med. Gaz.*, Vol. 82, No. 5, p. 247).
2. CHATTERJI, S. N., and DHARMENDRA.—Lagopthalmos in Leprosy (*Leprosy in India*, Vol. XIX, No. 2).
3. DAS GUPTA, C. R.—Value of Transfusion in the Treatment of Anæmia (*I.M.G.*, Vol. LXXXII, No. 6).
4. DAS GUPTA, C. R., and CHATTERJEE, J. B.—Hypoproteinæmia due to Utilization of Plasma Protein for Erythropoiesis (*I.M.G.*)
5. DESIKACHAR, H. S. R., DE, S. S., and SUBRAHMANYAN, V.—Studies on the Nutritive Value of Soya-bean Milk—Part I (*Annals of Biochem. & Exp. Med.*, 6, No. 2, p. 49).
6. DESIKACHAR, H.S. R., DE, S. S., and SUBRAHMANYAN, V.—Studies on the Nutritive Value of Soya-bean Milk—Part (*Annals of Biochem. & Exp. Med.*, Vol. 6, No. 2, p. 57).
7. DESIKACHAR, H. S. R., DE, S. S., and SUBRAHMANYAN, V.—Studies on the Nutritive Value of Soya-bean Milk—Part II (*Annals of Biochem. & Exp. Med.*, Vol. 6, No. 2, p. 62).
8. DE, S. S., DESIKACHAR, H. S. R., KARNAI, B. T., and SUBRAHMANYAN, V.—Investigations on Soya-bean Milk and related Products (*Sci. & Culture*, Vol. 12, p. 587).
9. DHARMENDRA, and MUKHERJI, N.—Lepromin Reaction in Subsided Lepromatous Cases (*Leprosy in India*, Vol. XIX, No. 1).
10. DHARMENDRA and JAIKARIA, S. S.—Failure to Sensitize presumably Non-leprous Individuals to Lepromin (*Leprosy in India*, Vol. XIX, No. 1).
11. DHARMENDRA, and MUKHERJI, N.—Treatment of Leprosy with Hydnocarpus Remedies (*Leprosy in India*, Vol. XX, No. 2).
12. DHARMENDRA.—The Lepromin Test—a Review (*Leprosy Review*, Lond.).
13. DHARMENDRA.—Leprosy in Ancient Indian Medicine (*International Journal of Leprosy.*)

14. GHOSH, L. M., DEY, N. C., and PANJA, D.—Studies of Ringworm Part IV, Incidence of *Trichophytom gypseum* in India (*Ind. Med. Gaz.*, Vol. LXXXII, No. 2, p. 73).

15. GOPALAN, C.—The Ætiology of 'Phrynoderma' (*Ind. Med. Gaz.*, Vol. LXXXII, No. 1, January, 1947).

16. PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS A. K.—Sulphaguandine in the Treatment of Cholera (*Ind. Med. Gaz.*).

17. PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS A. K.—Sulphadiazine in the Treatment of Cholera (*Ind. Med. Gaz.*).

18. PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS A. K.—Sulphasuzidine in the Treatment of Cholera (*Ind. Med. Gaz.*).

19. PASRICHA, C. L., PAUL, B. M., DAS GUPTA, A. C., and DAS A. K.—Phthalylsulphathiazole in the Treatment of Cholera (*Ind. Med. Gaz.*)

20. PATWARDHAN V. N., and DIKSHIT, P. K.—Studies in Experimental Rickets: The Alkaline Serum Phosphatase in Rachitic Albine Rats (Vol. 32(2) April, 1947, p. 91).

21. PANJA, D., DEY, N. C., and GHOSH, L.M.—Sporotrichosis of the Skin in India (A New Species described) (*Ind. Med. Gaz.*, Vol. LXXXII, No. 4, p. 200).

22. ROY, S. C., ROY, S. K., and GUHA, B. C.—Role of Thiamin and Riboflavin in the Biosynthesis of Vitamin C (*Nature*, 1946, 158, 238).

23. ROY, S. C.—Studies in Certain Aspects of the Biosynthesis of Vitamin C.

24. SAHASRABUDHE, M. R., DESIKACHAR, H. S. R., and LAKSHMINARAYAN RAO, M. R.—Biological Value of Soya-bean Milk Protein by Regeneration and Liver Protein (*Curr. Sci.*, October, 1947).

25. SUNDARARAJAN, A. R.—Vitamin A in Fish-Liver Oils and Carotene in Foodstuffs (*Curr. Sci.*, Vol. 16, No. 3, March, 1947).

26. Some Rich Sources of Active Carotenoids (*Jour. of Sci. & Ind. Res.*, July, 1947).

27. Studies in Nutritive Value of Cereals and Pulses (*in Press*).

VIII. PROGRAMME OF RESEARCHES AND MISCELLANEOUS GRANTS RECOMMENDED FOR THE YEAR 1948-49.

The following enquiries and budget allotments were recommended to the Governing Body by the Scientific Advisory Board at its meeting held on the 12th and 13th December, 1947.

Enquiries

		Budget allotments Rs.
	Cholera.	
	Continuation enquiries :—	
1.	Cholera treatment under the Director, School of Tropical Medicine, Calcutta	10,000
2.	Cholera treatment unit under the Director, King Institute, Guindy, Madras	30,967
3.	Enquiry on cholera under the Director, Central Research Institute, Kasauli	20,266
	Malaria.	
	Continuation enquiries :—	
4.	Insecticide and mosquito repellent enquiry under the Director, Malaria Institute of India, Delhi	43,599
5.	Enquiry on malaria under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta	6,900
6.	Enquiry on the control of sandflies *Phlebotomus argentipes* by spraying with DDT under Dr. D. N. Roy at the School of Tropical Medicine, Calcutta	4,000
	New enquiries :—	
7.	Enquiry on the chemotherapy of malaria under Dr. K. Ganapathi, Assistant Director, Department of Chemotherapy, Haffkine Institute, Bombay	21,074
	Nutrition.	
	Continuation enquiries :—	
8.	Nutrition Research Laboratories, Coonoor	1,85,554
9.	Nutrition research unit under Dr. R. G. Chitre at the Seth G. S. Medical College, Bombay	20,590
10.	Enquiry on the formation function and variations of plasma proteins in health and disease under Mr. N. C. Datta at the Grant Medical College, Bombay	4,951
11.	Enquiry on the evaluation of proteins in terms of haemopoietic activity and the relation of dietary protein to blood and muscle haemoglobin under Prof. M. Damodaran at the University Biochemical Laboratory, Triplicane, Madras	4,993
12.	Enquiry entitled "Preparation of antirachitic substances" under Prof. B. V. Bhide at the Maharajah Pratap Sinh Chemical Laboratory, S. P. College, Poona	10,690

	Enquiries.	Budget allotments Rs.
13.	Bangalore school-feeding scheme and the enquiry on the nutritive value of soya-been milk under Prof. V. Subramanyan and Dr. S. S. De at the Indian Institute of Science, Bangalore.	16,131
14.	Nutrition research unit under Professor V. Subrahmanyan and Dr. S. S. De at the Indian Institute of Science, Bangalore ..	5,478
15.	Enquiry on plant carotenoids in relation to their biological importance at the Laboratories of the Council of Scientific and Industrial Research, Delhi	7,404
16.	Enquiry on the growth-promoting factor present in Bengal gram at the Laboratories of the Council of Scientific & Industrial Research, Delhi	4,269
	New enquiries :—	
17.	Enquiry on the metabolism of vitamin 'A' under Dr. K. Rajagopal, Associate Professor of Nutrition at the All-India Institute of Hygiene & Public Health, Calcutta	5,734
18.	Enquiry on the role of fat in lactose metabolism under Dr. S. Banerjee, Diabetes Research Worker at the School of Tropical Medicine, Calcutta	5,480
19.	Nutrition research unit under Dr. B. C. Guha, Head of the Department of Applied Chemistry, University College of Science & Technology, Calcutta	25,000
	Leprosy.	
	Continuation enquiries :—	
20.	Leprosy enquiry under Dr. Dharmendra at the School of Tropical Medicine, Calcutta	17,196
21.	Enquiry entitled 'Study of histopathology of leprosy' under Dr. R. G. Cochrane, Honorary Director, Leprosy Campaign Madras	24,845
	Plague.	
	Continuation enquiries :—	
22.	Plague researches under the Director, Haffkine Institute, Bombay	23,472
23.	Plague enquiry at Coonoor in the Nilgiris District under the Director of Public Health, Madras	6,000
	Clinical Research.	
	Continuation enquiries :—	
24.	Clinical research unit under Dr. V. R. Khanolkar at the Tata Memorial Hospital, Bombay	14,346
25.	Neuropathological unit at the Tata Memorial Hospital, Bombay	48,110
26.	Enquiry on Naga sore under Dr. S. R. Pandit at the Pasteur Institute, Shillong	7,000

	Enquiries.	Budget allotments Rs.
27.	Enquiry on the role of nutritional factors in hepatic cirrhosis under Dr. M. V. Radhakrishna Rao at the Haffkine Institute, Bombay	12,317
28.	Enquiry on cirrhosis in animals and its bearing on the ætiology of infantile biliary cirrhosis under Dr. T. Bhaskara Menon at the Andhra Medical College, Vizagapatam	7,052
29.	Enquiry on the clinical and biochemical investigations on certain aspects of infantile hepatic cirrhosis under Profs. V. Subrahmanyan and V. R. Naidu at the Indian Institute of Science, Bangalore and at Sri Krishnarajendra Hospital, Mysore ..	10,955
30.	Enquiry on typhus under Dr. D. W. Soman at the Haffkine Institute, Bombay	13,500
31.	Enquiry into typhus in Bengal under Dr. K. V. Krishnan at the All-India Institute of Hygiene & Public Health, Calcutta ..	14,000
32.	Enquiry on the effect of vitamins B and C in diabetes under Dr. S. Banerjee, Diabetes Research Worker at the School of Tropical Medicine, Calcutta	10,000
33.	Research on lathyrism in the Central Provinces & Berar ..	17,000
34.	Hæmatological unit under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	10,535
35.	Helminthological enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta	8,813
36.	Enquiry to investigate allergic diseases and significance of endogenous allergens as regards ætiology, diagnosis and treatment of allergic diseases under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta	8,520
37.	Enquiry on the enteric group of fevers under Dr. R. N. Chaudhuri at the School of Tropical Medicine, Calcutta	5,452
	New enquiries :—	
38.	Enquiry on the immunological properties of lens and uveal proteins under Dr. S. N. Cooper at the Seth G. S. Medical College, Bombay	10,190
39.	Enquiry on the neurogenic factor in the production of traumatic shock and its treatment by the alteration of cerebro-spinal fluid electrolytes under Dr. P. K. Sen at the Seth G. S. Medical College, Bombay	8,440
40.	Enquiry into erythroblastosis foetalis and the Rh factor under Lieut.-Colonel S. D. S. Greval, Imperial Serologist to the Government of India, at the School of Tropical Medicine, Calcutta ..	5,540
41.	Enquiry to investigate bacteriocidal protistocidal and fungicidal action of phytencides under Dr. J. C. Ray, Director, Indian Institute for Medical Research, Calcutta	5,790
42.	Investigation into the chromatophorotropic hormone of the pituitary gland and its employment in the treatment of pig-	

ENQUIRIES.	Budget allotments Rs.
mentary disorders of the skin, e.g. leucodarma, vitiligo, etc., under Dr. B. Mukherji, Director, Central Drug Laboratory, Calcutta	28,490
Maternity and Child Welfare.	
Continuation enquiries :—	
43. Enquiry into the pelvic types and its relationship to body-build in Indian women under Doctors S. Mitra and S. Mukerjee at the Carmichael Medical College, Calcutta	2,500
44. Enquiry on the anæmias in pregnancy under Dr. R. G. Dhayagude at the Seth G. S. Medical College, Bombay	7,689
45. Enquiry into the reproductive patterns of urban and rural populations under Dr. C. Chandra Sekar, Professor of Statistics and Dr. Muktha Sen at the All-India Institute of Hygiene & Public Health, Calcutta	9,100
New enquiries : -	
46. Enquiry on anæmia in pregnancy under Dr. C. R. Das Gupta at the School of Tropical Medicine, Calcutta	7,200
Filariasis.	
Continuation enquiries :—	
47. Filariasis enquiry under Dr. A. K. Mukerji at the School of Tropical Medicine, Calcutta	7,644
48. Enquiry to test the usefulness of the intradermal and other immunological tests in the diagnosis of early filariasis under the Director, King Institute, Guindy, Madras	7,003
49. Enquiry on changing the physical properties of drugs by wetting agents under Dr. B. B. Dikshit, Principal, B. J. Medical College, Poona and Dr. K. V. Venkataraman, Director of Technology, Department of the University of Bombay	9,957
Industrial Health.	
Continuation enquiries :—	
50. Industrial health research unit at the All-India Institute of Hygiene & Public Health, Calcutta	39,634
51. Enquiry into environmental conditions such as lighting, temperature, humidity, ventilation and dust in certain industries in and around Calcutta under Professor K. Subrahmanyan, Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, Calcutta	6,010
52. Enquiry into sickness absenteeism due to accidents at the Bata Shoe Factory under the Chief Medical Officer, Batanagar	7,460
53. Enquiry on fatigue and efficiency in paper and textile industries under Professor Kali Prasad at the Laboratories of Experimental Psychology, Lucknow University, Lucknow	6,666

	ENQUIRIES.	Budget allotments Rs.
	New enquiries :—	
54.	Enquiry into the problem of traffic accidents and accident-prone personnel amongst bus and tram drivers in Calcutta under Mr. S. K. Bose, Lecturer in Industrial Psychology, Calcutta University, Calcutta	720
	Pharmacology.	
	Continuation enquiries :—	
55.	Indigenous drugs enquiry under Bt.-Colonel Sir Ram Nath Chopra, C.I.E., I.M.S.(*retd.*), at the Drug Research Laboratory, Jammu; Kashmir	8,398
	New enquiries :—	
56.	Indigenous drugs enquiry under Dr. J. C. Gupta at the School of Tropical Medicine, Calcutta	26,242
	Other Researches.	
	Continuation enquiries :—	
57.	Enquiry on filterable viruses under the Director, King Institute, Guindy, Madras	14,987
58.	Enquiry on the action of snake venoms and their constituents on the biochemical activity of the tissue cells under Dr. B. N. Ghosh at the University College of Science & Technology, Calcutta	4,604
	GRANTS.	
(i)	Office of the Secretary, Indian Research Fund Association ..	97,906
(ii)	Staff employed in the office of the Accountant-General, Central Revenues, for I.R.F.A. work	10,000
(iii)	I.R.F.A. publications	7,000
(iv)	Travelling allowance of the members of the Governing Body, Scientific Advisory Board and Advisory Committees of the I.F.R.A.	18,000
(v)	Cost of two professorships at the School of Tropical Medicine, Calcutta	29,185
(vi)	Contribution to the Imperial Institute of Entomology, London	1,350
(vii)	Contribution towards the Bureau of Hygiene & Tropical Diseases, London	2,025
(viii)	Research fellowships	62,500
(ix)	*Indian Journal of Medical Research* and *Indian Medical Research Memoirs*	
	(a) Printing and publication charges of the *Journal* 14,000	
	(b) Printing and publication charges of *Memoirs* 2,000	
	(c) Editor's office 9,493	25,493

	Enquiries		Budget allotments Rs.
(x)	*Indian Journal of Malariology*		
	(a) Printing and publication charges of the *Journal*	10,000	
	(b) Editor's office	3,420	13,420
(xi)	I.R.F.A. Library at the Central Research Institute, Kasauli		2,530
(xii)	I.R.F.A. microfilm service unit		11,814
(xiii)	Pay (including special pay, overseas pay and allowances) and contributions towards leave, salary and pension or contributory provident fund of officers of the Medical Research Department under the Association		75,000
(xiv)	Travelling allowances and certain contingent expenses for Medical Research Department officers who are employed under the I.R.F.A. and are attached to central or provincial laboratories		10,000
(xv)	Pay and travelling allowance of Junior Research Worker ..		10,880
(xvi)	Clerical assistance at the School of Tropical Medicine, Calcutta		6,674
(xvii)	Clerical assistance at the All-India Institute of Hygiene & Public Health, Calcutta		3,361
(xviii)	Travelling allowance for I.R.F.A. workers on transfer ..		3,000
(xix)	I.R.F.A. contribution and interest towards the Contributory Provident Fund		25,000
(xx)	Miscellaneous unforeseen expenditure		60,000
(xxi)	Provision for a mobile unit for clinical research		33,460
(xxii)	Provision for increase in pay, dearness allowance, compensatory and house-rent allowances at the revised rates ..		40,000

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on 01 February, 2020